IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Wedel et al.
Appl. No.	:	10/777,838
Filed	:	February 12, 2004
For	:	COMPOSITIONS AND METHODS FOR TREATMENT OF POUCHITIS
Examiner	:	Dana H. Shin
Group Art Unit	:	1635
Confirmation No.	:	5903

DECLARATION OF DR. BRUCE R. YACYSHYN UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Bruce R. Yacyshyn, M.D., based on personal knowledge or information, declare and state as follows:

1. I am a Professor of Clinical Medicine in the division of Digestive Diseases at University of Cincinnati College of Medicine. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

2. I am an expert in the field of inflammatory bowel diseases, with over 22 years of experience in the field as both a clinician and research scientist. I have reviewed the above-captioned application and the currently pending claims. I have extensive knowledge of the subject matter of the above-captioned application, including the treatment of Crohn's disease (CD), ulcerative colitis (UC), and pouchitis, as well as the antisense compound known as alicaforsen (ISIS 2302). I am informed that the time of the invention was not later than February 13, 2003. I have served as a paid Medical Advisor for Isis Pharmaceuticals, Inc., the assignee of the above-captioned application.

3. I have reviewed the Office Action dated February 17, 2011 and all of the references cited by the Examiner in that Office Action. I have reviewed the Examiner's arguments and conclusion that a person of ordinary skill in the art would have found the claimed invention obvious at the time the invention was made. In particular, the Examiner concludes that one of skill in the art would have had a reasonable expectation of success in treating pouchitis with an enema formulation of antisense directed to ICAM-1 (ISIS 2302). I have also reviewed the Examiner's arguments and conclusion that the results reported in Example 17 of the patent

demonstrating remission of 58% of patients were reasonably expected at the time the invention was made. For the reasons detailed below, it is my opinion that both of these conclusions are wrong.

4. At the time of the invention, one of ordinary skill in the art would not have had a reasonable expectation that treating pouchitis with an enema formulation of ISIS 2302 would have been successful in view of the cited references. The group of diseases generically referred to as inflammatory bowel diseases (IBD) are a diverse group of distinct diseases believed to have distinct causes. *See, e.g., Yacyshyn and Pilarski*, Gut 1993, 34:1698-1704; *Yacyshyn*, Imm. Cell Bio. 1993, 71:265-274; *Lawrance et al.*, Human Mol. Gen., 2001, 10:445-456 (Exhibits B-D). While inflammatory bowel diseases do have shared commonalities (*e.g.*, inflammation of the bowel) which are the basis for their being grouped together, that does not mean that there was an expectation that treatments for CD, UC and pouchitis were interchangeable. To suggest that treatments for CD or UC would be expected to work for pouchitis because all three are inflammatory bowel diseases is similar to arguing that treatments for Parkinson's or Huntington's disease would reasonably be expected to work for Alzheimer's disease because they are all "neurodegenerative disorders" characterized by neuronal death.

5. Pouchitis is a disease of the ileal pouch. Some patients with severe UC and familial adenomatous polyposis undergo surgery in which the colon is removed, and an artificial pouch is made out of the distal portion of the small intestine as a substitute for the colon. *See, e.g., Sandborn et al.* Mayo Clin. Proc. 1994, 69:409-415 at page 409; *Sandborn et al.*, Trends in Inflam. Bowel Dis. Ther. 1996, at page 52. (Exhibits E and F). This pouch sometimes develops inflammation known as pouchitis. *Achkar and Shen*, Cur. Gastro. Rep. 2001, 3:484-490 at page 484 (Exhibit G).

6. While similarities between pouchitis and UC have led some to speculate that pouchitis may be a recurrence of UC, (*see, e.g., Schouten*, Mediators Inflamm. 1998, 7:175:181 (Exhibit H)), the generally accepted view was and continues to be that pouchitis is a disease of unknown origin distinct from UC. *See, e.g., Sandborn et al.* at page 61; *Mahadevan and Sandborn*, Gastroent. 2003, 124:1636-1650 (Exhibit I); . That all pouchitis is not a reoccurrence of UC is evident from the fact that pouchitis can develop in patients that have never had UC, for example, patients with familial adenomatous polyposis. *See Achkar and Shen* at page 484. In addition, were it generally accepted that pouchitis is a reoccurrence of UC, the first line treatment for pouchitis would be the same as that for UC – it is not. UC was and continues to be treated primarily by anti-inflammatory drugs, steroids and immuno-suppressants, while pouchitis is treated with antibiotics or probiotics. *See, e.g., Ghosh et al.*, BMJ 2000, 320:1119-1123 (Exhibit J); *Wolf and Lashner*, Cleveland Clinic J. Med. 2002, 69:621-631 (Exhibit K); *Achkar and Shen* at page 489; *Shen and Lashner*, Gast. Hep. 2008, 4:355-361 at page 359 (Exhibit L).

7. Pouchitis is also not the same as Crohn's disease, which is a <u>systemic</u> disease with inflammation primarily in the intestine. *See e.g., Yacyshyn*, Imm. Cell Bio.; *Subramani et al.*, Gut 1993, 34:1539-1542 (Exhibit M). The first line treatment for CD is not the same as pouchitis – CD was and continues to be treated primarily with anti-inflammatory compounds and immuno-suppressants. *See e.g.*, *Wolf and Lashner* at 629; *Scribano and Prantera*, Aliment. Pharmacol. Ther. 2002, 16 (Supp. 4):35-39 (Exhibit N); *Mahadevan and Sandborn* at page 1644. In rare instances of misdiagnosis, patients with an inflamed ileal pouch can be initially diagnosed as

diagnosed as having pouchitis, only to be later correctly diagnosed with CD of the pouch. *See, e.g., Svaninger*, Scand. J Gastro. 1993, 28:695-700 at page 699. Such a misdiagnosis is extremely unfortunate, as it can lead to life-threatening delays in proper treatment for CD, which treatment is distinct from pouchitis. *See, e.g., Mahadevan and Sandborn* at page 1644; *Achkar and Shen*. It is important to note that patients with CD are rarely intentionally treated by the formation of an ileal pouch since CD can affect the entire digestive tract, including the portion of the small intestine used to form the pouch, leading to active CD in the pouch. *See., e.g., Subramani et al.*, at page 1542.

8. Thus, it is my opinion that at the time of the invention, and currently, one of skill in the art would have recognized that although CD, UC and pouchitis are similar in some respects, they are distinct diseases with different standard treatments, and there was no reasonable expectation that what was successful in treating CD or UC would be successful in treating pouchitis. This understanding applied to treatments generally, as well as to the claimed method of treating pouchitis with an enema containing ISIS 2302, specifically.

9. This is not to say that there were no treatments which were hypothesized or known to work for all three diseases. The Examiner has cited three references which purportedly disclose treatment methods that are useful to treat all three diseases: *Sandborn et al.* (U.S. Patent No. 5,846,983), *Sachetto et al.* (U.S. Patent No. 7,341,741) and *Kono et al.* (6,730,702). The Examiner concludes that these references establish that one can treat pouchitis with the same methods used to treat CD or UC. I have reviewed these references, and it is my opinion that these references do not support this conclusion for the following reasons. Firstly, as discussed above, pouchitis can occur independently from IBD and is not therefore an extension of IBD but an independent, unique disease process. Secondly, pouchitis is not treated like other forms of IBD – as discussed above, first line therapy for this condition is antibiotic administration, unlike CD and UC where antibiotic administration in numerous studies has not been shown to be beneficial. In addition, the cited references do not offer any support for the Examiner's conclusion as detailed below.

10. *Sandborn et al.* (U.S. Patent No. 5,846,983) state that they have developed a method of treating IBD by locally administering nicotine. However, the *Sandborn* reference does not contain any data demonstrating treatment of any of the three diseases. Therefore, it is my opinion that this reference does not support the Examiner's argument, as it constitutes nothing more than educated speculation which is not supported by any evidence. Similarly, *Sachetto et al.* also assert that they developed a treatment for IBD by administering xanthan gum or HPMC. But there is only a single example, in which xanthan gum enemas are used to treat pouchitis – there are no examples of treating UC or CD patients. Therefore, it is my opinion that this reference also does not support the Examiner's argument, as it too constitutes speculation which is not supported by sufficient evidence. Finally, the Examiner relies on *Kono et al.*, which discloses anecdotal evidence of the use of the anti-ulcer drug ecabet sodium to treat CD, UC and pouchitis. The reference discloses treatment of a single patient with CD, three patients with UC, and a single patient with pouchitis. It is my opinion that this limited evidence is not sufficient to establish a general expectation that a compound which successfully treats CD, UC or both will also successfully treat pouchitis. Nor is it sufficient to reasonably establish the specific expectation that an enema comprising ISIS 2302 could successfully treated pouchitis, as ecabet sodium is not an antisense compound and is not known to regulate ICAM-1 levels. For the

reasons discussed above, an expert with ordinary skill in the field would not have reasonably expected that a treatment which was successful at treating CD, UC or both would also successfully treat pouchitis. It is my opinion that the references relied on by the Examiner, alone or in combination, are not sufficient to overcome this general understanding in the art at the time of the invention.

11. The Examiner concludes that it was accepted by experts in the field at the time the invention was made that the underlying cause for UC and pouchitis are commonly shared, and that UC and pouchitis were closely interrelated. The Examiner relies on *Svaninger et al.* and *Patel et al.* to support this conclusion. I have reviewed *Svaninger et al.* and it is my opinion that it does not support the Examiner's conclusions. The *Svaninger* reference was published in 1993. The single statement speculating that pouchitis may be caused by a genetic component shared with UC patients is not sufficient to support the Examiner's conclusion in view of subsequent papers, including those referenced above, which have established that UC and pouchitis are most likely distinct diseases. In addition, as noted above, the statement in *Svaninger* that the diagnosis of pouchitis in four patients subsequently proved to be CD indicates that pouchitis and CD are distinct diseases, not that pouchitis and CD are related. *See Svaninger* at page 699, first full paragraph.

12. Similarly, it is my opinion that the observation in *Patel et al.* that serum ICAM-1 is elevated in active CD, UC and pouchitis is not sufficient to establish that the three diseases are closely interrelated. It was well-known at the time of the invention that ICAM-1 and related cell adhesion molecules are markers of inflammation generally, and therefore are elevated in a number of unrelated diseases, including chronic inflammatory liver disease, diabetes, some carcinomas, allograft rejection and systemic vasculitides. *See Patel* at page 1037. Thus, the presence of elevated ICAM-1 in active UC and pouchitis does not establish that they are closely interrelated any more than it establishes that UC and diabetes are closely interrelated. The speculation by *Patel et al.* that pouchitis might represent a reactivation of the immunological mechanism underlying UC has not been borne out by subsequent research. As discussed above, the generally accepted view in the field is that UC and pouchitis are distinct diseases. Finally, it is my opinion that the statement by *Patel et al.* that inhibition of leucocyte-endothelial cell interaction could, hypothetically, provide a new target for control of IBD is nothing more than an untested hypothesis. Such an untested hypothesis was not sufficient to provide a reasonable expectation of success of treating pouchitis by inhibiting ICAM-1 expression to someone working in the field as argued by the Examiner.

13. I am the lead author on two of the references relied on by the Examiner: *Yacyshyn et al.* (1999 and 2002). It is my opinion that the treatment of CD using intravenous administration of ISIS 2302 reported in *Bennett et al.* and *Yacyshyn et al.* (1999 and 2002) does not provide a reasonable expectation that an enema formulation of ISIS 2302 could treat pouchitis. As discussed above, CD and pouchitis were recognized by experts in the field as distinct diseases. CD was, and continues to be, thought of as a systemic autoimmune disorder, while pouchitis was viewed as a local inflammation of the ileal pouch. Therefore, treatment of CD by i.v. administration of ISIS 2302 was not viewed by those in the field as providing a reasonable expectation of successfully treating pouchitis by enema administration of ISIS 2302. Nor does the disclosure of the possible treatment of UC by administration of ISIS 2302 provide a reasonable expectation that an enema formulation of ISIS 2302 could treat pouchitis. None of

these references disclose the actual treatment of UC using ISIS 2302. And, even if they did, UC and pouchitis were recognized by experts in the field as distinct diseases, and there was no reasonable expectation in the field that treatments for UC would successfully treat pouchitis, as discussed above.

14. For the reasons stated above, it is my opinion that at the time of the invention, there was no reasonable expectation that an enema formulation of an antisense oligonucleotide having SEQ ID NO: 1 could successfully treat pouchitis. It is my opinion that, for the reasons discussed above, the references relied on by the Examiner, considered individually and in combination, do not support the conclusion that one of ordinary skill in the field would have had a reasonable expectation that the claimed methods would be successful.

UNEXPECTED RESULTS

15. It is my opinion that the results reported in Example 17 of the specification are unexpected in view of what was known at the time of the invention, including the references relied on by the Examiner. None of the references relied on by the Examiner, including *Bennett et al.* and *Yacyshyn et al.* (1999 and 2002) report any data demonstrating the treatment of any IBD other than CD using ISIS 2302. It is therefore factually incorrect to state, based on these references, that at the time the invention was made it was an art-recognized fact that ISIS 2302 was capable of producing remission in any IBD patient other than a CD patient. For the reasons stated above, treatments for CD were not viewed as predictive of treatments for pouchitis. Therefore, the successful treatment of CD by i.v. administration of ISIS 2302 would not lead an expert in the field to expect that ISIS 2302 would successfully treat pouchitis as reported in Example 17. Nor does the treatment of pouchitis using tixocortol pivalate (*Karp, et al.*, Digest. Dis. and Sci., 33(3):85S-87S (1988)), ecabet sodium (*Kono et al.*), nicotine (*Sandborn 1998*) or xanthan gum enemas (*Sachetto*) provide any expectation of the results reported in Example 17, as one of skill in the art would not expect the success of ICAM-1 antisense based on the success of compounds unrelated to ICAM-1 antisense.

16. It is particularly unexpected that the method described in Example 17 was able to achieve remission of 58% of patients at the end of treatment, and remission of 50% of the patients one month after treatment, since the patients were suffering from chronic, unremitting pouchitis that was unresponsive to conventional therapies. *Bennett et al.* report that 47% CD patients were in remission following treatment, and *Yacyshyn et al.* (2002) disclose that only 41% of CD patients experienced remission at the end of treatment, for a combined average remission rate of only 44%. As discussed above, it is my opinion treatments for CD are not interchangeable with treatments for pouchitis, and therefore these results are not directly comparable. In addition there are differences in the intensity of the disease, drug co-therapy, the amount and route of antisense administration, and length of treatment. However, comparing the results of Example 17 to those of *Bennett et al.* and *Yacyshyn et al.* (2002), the results of Example 17 are unexpectedly superior.

Appl. No. : **10/777,838**
Filed : **February 12, 2004**

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: Bruce R. Yacyshyn Date: 16 Aug 2011

Bruce R. Yacyshyn, M.D.

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Exhibit A

– CURRICULUM VITAE –

BRUCE R. YACYSHYN, MD, FRCPC, FACG

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MEDICAL LICENSURE
1986 Missouri, R9F93
1989 Alberta, 3927
2001 Ohio, 35.080517
2006 British Columbia, 27065
2008 Indiana, 01064973A

A. Positions and Honors

Positions and Employment

1982-1983	Chief Intern, Grey Nuns Hospital, Edmonton, Alberta, Canada
1983-1985	Medical Residency, Internal Medicine, University of Toronto, Toronto, Ontario
1985-1986	Senior Medical Resident, Internal Medicine, Sunnybrook Medicine Centre, Toronto, Ontario, Canada
1986-1989	Clinical and Research Fellow and Clinical instructor in Gastroenterology, Washington University School of Medicine, St. Louis, Missouri
1989-1994	Assistant Professor, Department of Medicine, Division of Gastroenterology, University of Alberta, Edmonton, Alberta, Canada
1995-2002	Associate Professor, Department of Medicine, Division of Gastroenterology, University of Alberta, Edmonton, Alberta, Canada
2000 -2002	Cross-Appointment, Department of Medical Microbiology and Immunology University of Alberta
2002-2005	Chief of Gastroenterology, Louis Stokes VAMC, Cleveland OH
2002-2005	Associate Professor of Medicine and Immunology, Case Western Reserve University School of Medicine
2005-2008	Medical Director and GI Category Leader, Procter and Gamble Pharmaceuticals, Cincinnati, OH
2009-current	Professor of Medicine, University of Cincinnati, Cincinnati, OH

Other Experience and Professional Memberships

1980-	Canadian Society for Immunology
1986-	American Gastroenterology Association
1989-	Canadian Association of Gastroenterology
1989-	Society for Mucosal Immunology
1992-	Canadian Society for Clinical Investigation
1998-	Medical Advisor ISIS Pharmaceutical, Carlsbad, California
1999-	American College of Gastroenterology, Fellow (2006)
1999-2002	Teaching Improvement for physicians and students (TIPS) instructor, CME Division, University of Alberta, Edmonton, AB, Canada
2002-	Crohn's and Colitis Foundation of America (CCFA) Professional Member
2005-2008	Medical director for Ascend III and Maintenance studies, Pediatric Asacol study,and Aryx IBS project at P & G Pharm.

Consultant Gastroenterologist for Libertas project, Ascend III biomarker team, OTC (over the counter) products and IAMS animal nutrition projects at P & G

Global regulatory and clinical investigator/research lead for the study of 800 mg tablet formulation for the Ascend III trial at P & G Pharm Outcome: The ASCEND III trial resulted in approval of an NDA (New drug application) with the FDA in 2009.

2007- Committee member Intraobvserver variation study for severity of Ulcerative Colitis (IOV) P &G Pharm.

2008 Patent obtained with P & G Pharmaceuticals Asacol treatment for IBS-d (with Dr. Simon Magowan) IPC8 class AA61K4500F1, USPC class 424 934, Patent application number 20090274662.

Global lead investigator/Medical Monitor for P & G Pharm on RDP-58 (a study of an oral anti-TNF inhibitor in Ulcerative Colitis)

2010 Principal investigator and consultant, Procter and Gamble: Project Cobalt. A single treatment consumer study (Concept and Use) in Normal Healthy Men and Women with Frequent Heartburn. Protocol number US109440

2010- PI at University of Cincinnati, a site for national clinical trial studying Vaccine for *Clostridium difficile* (Sanofi-Aventis)

2010- PI for research project: Cell Mediated Immunity in Clostridium difficile in Recurrent patients. Funded for 2 years (Merck)

Honors

1987-1989 Research Fellow, Alberta Heritage Foundation for Medical Research

1989 American Gastroenterological Association Senior Fellowship Research Award

1996 Finalist, Canada's Top 40 under 40

B. Selected Peer-reviewed Publications

1. Pilarski LM, **Yacyshyn BR**, Jensen GS, Pruski E, Pabst HS. β1 integrin (CD29) expression on human postnatal T-cell subsets defined by selective CD45 isoform expression. Journal of Immunology 1991; 147(3): 830-837.
2. **Yacyshyn BR** and MacLean GD Tumor markers in gastrointestinal malignancy: What use to the clinician? Canadian Journal of Gastroenterology 1992. 6(6): 329-333.
3. **Yacyshyn BR** and Pilarski LM. Expression of CD45RO on circulating CD19+ B-cells in Crohn's disease. 1993 GUT 34:1698-1704.
4. **Yacyshyn BR** Activated CD19 population in ulcerative colitis lamina propria mononuclear cells. Journal of Immunology and Cell Biology 1993 71:265-274.
5. Jensen GS, Belch RB, Mant MJ, Reuther BA, **Yacyshyn BR**, Pilarski LM. Expression of multiple 1 integrins on circulating monoclonal B-cells in patients with multiple myeloma. American Journal of Hematology 1993 43:29-36.
6. **Yacyshyn BR**, Cheung CM, Gordon-Malkin D, Pappas SC. A potential prognostic indicator of fulminant hepatic failure: A valuable adjunct in pre-liver transplant assessment? Canadian Journal of Gastroenterology 1994 8(5):308-312.
7. **Yacyshyn BR**, Lazarovits A, Tsai V, Matejko K. Crohn's disease, ulcerative colitis and normal intestinal lymphocytes express integrins ina dissimilar pattern. Gastroenterology 1994 107:1364-1371
8. **Yacyshyn BR**, Yacyshyn EA. Irritable bowel syndrome: A multiplex enteric response. Prairie Medical Journal 1994 64(3):97-9.
9. Pilarski LM, **Yacyshyn BR**, Lazarovits AI. Analysis of peripheral blood lymphocyte populations and immune function from children exposed to cyclosporine or to Azathioprine in utero. Transplantation 1994 57(1): 133-144.
10. **Yacyshyn BR** and Meddings JB. CD45RO expression on circulating CD19+ B-cells in Crohn's disease correlates with intestinal permeability. Gastroenterology 1995 108:132-137.
11. **Yacyshyn BR**, Longenecker BM, Biermann WA, McClure D, Poppema S, Bowen-Yacyshyn MB Active specific immunotherapy in the management of adenocarcinoma of the pancreas. The Canadian Journal of Gastroenterology 1995 9(4): 213-216.

12. **Yacyshyn BR**, Pilarski LM, Bowen-Yacyshyn MB. Inhibition by Rapamycin of P-glycoprotein 170-mediated export from normal lymphocytes:Implications for immunosuppression protocols. Scandinavian Journal of Immunology 1996 43:449-445.
13. **Yacyshyn BR**, Meddings J, Sadowski D, Bowen-Yacyshyn MB. Multiple sclerosis patients have peripheral blood CD45RO+ B-cells and increased Intestinal permeability. Digestive Diseases and Sciences 1996 41(12):2493-2498.
14. **Yacyshyn BR**, Multidrug resistance in inflammatory bowel disease 1997. International Review of Allergy & Clinical Immunology 3(1):64-69.
15. **Yacyshyn BR**, Bowen-Yacyshyn MB, Jewell L, Tami JA, Bennett CF, Kisner DL, Shanahan WR A placebo-controlled trial of ICAM-1 antisense oligonucleotide in the treatment of Crohn's disease. Gastroenterology 1998; 114:1133-1142.
16. **Yacyshyn BR**, Maksymowych W, Bowen-Yacyshyn MB. Differences in P-glycoprotein-170 expression and activity between Crohn's disease and ulcerative colitis. Human Immunology 1999; 60:677-687.
17. Chang Q, Soper B, **Yacyshyn BR**, Tepperman BL. Alterations in protein kinase C isoforms in experimentally-induced colitis in the rat. Inflammatory Research 1999; 48:1-9.
18. **Yacyshyn BR** and Thomson ABR. A critical review of acid suppression in non-variceal, acute, upper gastrointestinal bleeding. Digestive Diseases 2000; 18:117-128.
19. **Yacyshyn BR**. Novel biological anti-inflammatory drugs for inflammatory bowel disease. Seminars in Colon & Rectal Surgery, Editor: D. Schoetz Jr., 2001; 12: (No 1):29-37.
20. **Yacyshyn BR** and Crooke ST. The concept and application of antisense oligonucleotides. Diseases of the Colon and Rectum. 2001; 44: (No 9) 1241-1243.
21. Bowen-Yacyshyn MB, Bennett CF, Stecker K, Nation N, Rayner D, Shanahan Jr WR, Taurog JD, **Yacyshyn BR**. Antisense to ICAM-1 (ISIS 9125) affects adhesion molecule expression of HLA-B27/β2 microglobulin trangenic rats. Journal of Pharmacology and Experimental Therapeutics. 2002; 302: 908-917.
22. **Yacyshyn BR,** Chey WY, Goff J, Salzberg B, Baerg R, Buchman AL, Tami J, Yu R, Gibiansky L, Shanahan W. Double-blind, placebo-controlled trial of the remission-inducing and steroid-sparing properties of an ICAM-1 antisense oligodeoxynucleotide (ISIS 2302) in active, steroid-dependent Crohn's disease Gut 2002;51: 30-36.
23. **Yacyshyn BR,** Barish C, Goff J, Dalke D, Gaspari M, Yu R, Tami J, Sewell KL. Dose Ranging, Pharmacokinetic Trial Of His Dose Alicaforsen (ICAM-1)Antisense Oligodeoxynucleotide)ISIS 2302 in Active Crohn's Disease. Alimentary Pharmacology and Therapeutics. 2002; 1-10.
24. **Yacyshyn BR** Thomson ABR The Clinical importance of proton pump inhibitor pharmacokinetics. Digestion. 2002;66:67-78.
25. **Yacyshyn,BR ,** Pilarski LM. Discussion on the Safety of 6-Mercaptopurine for Childbearing Patients with Inflammatory Bowel Disease:A Retrospective Cohort Study . Gastroenterology,2003; 125:1562-c.
26. **Yacyshyn, BR**, Schievella A, Sewell KL, Tami JA Gene polymorphisms and serological markers of patients with active Crohn's disease in a clinical trial of antisense to ICAM-1. Clinical and Experimental Immunology; 2005 141:141-147.
27. Mant, MJ, Bain,VG, Maguire,CG, Murland,K, **Yacyshyn, BR,** Prevalence of occult gastrointestinal bleeding in celiac disease. Clinical Gastroenterol Hepatol.; 2006 4:451-4.
28. **Yacyshyn B,** Chey WY, Wedel MK, Yu RZ, Paul D, Chuang E. A randomized, double-masked, placebo-controlled study of alicaforsen, an antisense inhibitor of intercellular adhesion molecule 1, for the treatment of subjects with active Crohn's Disease. Clin. Gastroenterol. Hepatol.; 2007 5(2): 215-20.
29. Hanauer SB, Sandborn WJ, Dallaire C, Archambault A, **Yacyshyn B**, Yeh C, Smith-Hall N. Delayed-release oral mesalamine 4.8g/day (800 mg tablets) compared to 2.4 g/day (400 mg tablets) for the treatment of mildly to moderately active ulcerative colitis: The ASCEND I trial. Can. J. Gastroenterol. 2007,21(12): 827-34.
30. **Bruce R Yacyshyn** Adhesion Molecule Therapeutics in IBD. 2008 Inflammatory Bowel Dis. 14 (S2): S279-80.
31. **Bruce R Yacyshyn** Adhesion Molecule Therapeutics in IBD. 2009 Inflammatory Bowel Dis. 14 (S2): S279-80.
32. Sandborn WJ, Regula J, Feagan BG, Belousova E, Jojic N, Lukas M, **Yacyshyn B**, Krzeski P, Yeh CH, Messer CA, and Hanauer SB. Delayed-release oral mesalamine 4.8 g/day (800-mg tablet is effective for patients with moderately active ulcerative colitis. Gastroenterology. 2009 Dec;137(6):1934-43.e1-3. Epub 2009 Sep 18.

Exhibit B

Gut 1993; 34: 1698–1704

Expression of CD45RO on circulating CD19+ B-cells in Crohn's disease

B R Yacyshyn, L M Pilarski

Abstract

Crohn's disease is an immunoregulatory disorder of the intestine that can be associated with systemic manifestations. This study analysed B-cell differentiation antigens to identify B-cell subpopulations unique to patients with Crohn's disease. CD45 isoform expression was used as an indicator of B-cell differentiation stage. This work shows that B-cells in blood and gut of patients with Crohn's disease are at an advanced stage of differentiation based on their unusual presentation of transitional (RA+ RO+) and late stage (RO+) CD45 isoforms on lamina propria lymphocytes, whereas normal intestinal lamina propria lymphocytes B-cells express primarily CD45RA. Crohn's disease patients had heightened expression of the CD45RO isoform on CD19+ lamina propria lymphocytes, and was found in a statistically significant proportion of Crohn's peripheral blood mononuclear cells (PBMC) where CD19+ PBMC had an expression pattern affecting an unexpectedly high proportion of these differentiated or late stage CD45RO+ B-cells. The expression of CD45RO varied greatly among CD19+ PBMC from patients with Crohn's disease, so multiple regression analysis was performed between these CD45 isoforms and several clinical parameters. After grouping high and low CD45RO expression on CD19+ B-cells, a significant statistical difference was found between high Crohn's disease activity index (CDAI) and low CDAI Crohn's disease patients respectively.

(*Gut* 1993; 34: 1698–1704)

The inflammatory bowel diseases Crohn's disease and ulcerative colitis are intestinal disorders of unknown causes. The clinical presentations of these two illnesses can be so similar as to prohibit their differentiation pathologically in a number of patients.[1] Specific aberrations of the mucosal immune system have been identified in inflammatory bowel disease (IBD) patients. For instance, a predominance of CD4+ and lymphokine activated killer lymphocytes together with a comparative deficiency of CD8+ cells has been found among Crohn's lamina propria lymphocytes.[2] This same study identified a decrease in CD45RA+ CD4+ in lamina propria lymphocytes compared with peripheral blood mononuclear cells (PBMC). Lamina propria lymphocytes from patients with IBD have been found to have heightened expression of activation markers (including transferrin receptor, IL-2 receptor and 4F2).[3-5] Humoral immune abnormalities in IBD affect various autoantibodies including rheumatoid factor, anti-colon antibodies, and neutrophil autoantibodies disputedly secondary to the underlying mucosal inflammation and not a primary pathophysiological factor.[6-8]

In this study we analysed the phenotypes of B and T cells from patients with IBD, with an emphasis on changes in the B-cells populating the lamina propria in comparison with those in PBMC, emphasising the selective expression of CD45 isoforms as a marker for differentiation within the B-cell lineage (reviewed in ref 9) and analysis of CD5 and CD11b, which have been associated with autoimmune B-cells or the activation state of the B-cell.[10] CD45, the leucocyte common antigen, is the most prevalent antigen on the surface of B and T lymphocytes and through its cytoplasmic domain, the tyrosine phosphatase activity plays a key part in intracellular signalling.[11] The CD45 isoforms, CD45RA and CD45RO are distinguished by differences in molecular weight, glycosylations and are encoded by alternatively spliced mRNA.[12-14] These isoforms characterise T-cell differentiation with the transition from expression of high molecular weight CD45RA (p220) isoform on naive cells to the low molecular weight CD45RO (p180) isoform on memory T-cells.[15,16] A recent study of this group of cell surface molecules in normal human intestinal mucosal CD3+ T-cells has shown that the intraepithelial lymphocyte population expressed mainly CD45RO.[17] CD3+ CD45RO+ T-cells are probably memory T-cells consistent with their role as primary immune regulators of an antigen bombarded environment such as the gut. B-Cells are also found, however, among the mucosal lymphocytes in normal and disease states. We have recently identified among B-cells a similar transition in CD45 isoforms to that found in T-cells.[9,18,19] Pre B cells express exclusively CD45RA at low density with an increase in CD45RA density as differentiation proceeds towards mature B-cell function (reviewed in ref 9). A transition from CD45RA to CD45RO expression seems to occur on in vivo antigen stimulated B-cells, which has been confirmed by in vitro studies.[18,19] Early plasma cells express only the low molecular weight CD45 isoforms, while end stage plasma cells eventually lose all CD45 expression.[19]

In this study, PBMC or lamina propria lymphocytes, or both have been characterised in Crohn's disease, ulcerative colitis (UC), coeliac sprue, and normal patients. Crohn's PBMC were studied by multiple regression analysis in relation to a number of clinical parameters corresponding to the patients studied. From our findings in this study, we show that analysis of CD45 isoforms can identify subpopulations with possible functional significance, of B and T

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lymphocytes in lamina propria lymphocytes and PBMC.

Materials and methods

PATIENTS AND CONTROLS

Specimens of the colon from patients with Crohn's disease and ulcerative colitis were obtained with the assistance of a pathologist to sample disease affected mucosa. Normal mucosa was obtained from the colon at least 15 cm distal to carcinoma in patients having resection as well as from normal areas in patients having resection for diverticulosis. Normal colon was also obtained from decreased persons donating organs for transplantation. A total of 10 'normal', eight Crohn's disease, and four ulcerative colitis colons were analysed. Peripheral blood was analysed from patients with Crohn's disease (n=33), as well as patients with ulcerative colitis (n=11), coeliac sprue (n=13), and normal Red Cross blood donor controls (n=9).

Many of the patients with Crohn's disease were enrolled in the MRC Canadian Crohn's Relapse Prevention Trial, and all had diagnoses established clinically and through pathological and radiological criteria. These patients were receiving various drugs, but primarily 5-acetylsalicylic acid and sulphasalazine. Patients studied were not receiving immunomodulatory drugs. Protocols were approved by the ethics committee of the Faculty of Medicine, University of Alberta.

PERIPHERAL BLOOD MONONUCLEAR CELL ISOLATION

PBMC were obtained with informed consent from 33 patients with Crohn's disease, as well as patients with UC, coeliac sprue, and normal Red Cross blood donors. PBMC were purified by centrifugation over Ficoll Paque (Pharmacia, Dorval, PQ) followed by two washes.

ISOLATION OF LAMINA PROPRIA LYMPHOCYTES

Normal large bowel intestinal mononuclear cells were obtained from surgically removed specimens from subjects donating organs for transplantation, from patients with diverticulosis, as well as from morphologically normal areas of large bowel at least 15 cm distal to diseased areas of colons resected for adenocarcinoma. Crohn's intestinal mononuclear cells were obtained from colonic specimens from patients having resections done for therapeutic reasons. UC intestinal mononuclear cells were similarly obtained from the colons of patients having therapeutic resections. Intestinal mononuclear cells were isolated from mucosa according to a well established protocol as follows.[5 20-22] Briefly, specimens were washed in RPMI media, the mucosa then dissected from submucosa. Mucosa was then cut into small 0·5 cm×0·5 cm minced pieces and washed repetitively in Hanks's balanced salt solution without calcium and magnesium containing antibiotics 1 mg/ml Ticarcillin (Beecham, Pointe-Claire, Canada), 0·5 mg/m Amikacin (Bristol Labs, Ottawa, Canada), 0·4% Septra (Burroughs-Wellcome, Kirkland, Canada), 2 mg/ml Fungizone Grand Island Biological Co (Gibco, Grand Island, USA), 10 mm hydroxyethyl piperazine-ethane sulphonic acid (HEPES), and NaOH to adjust to pH 7·4. The minced pieces were stirred in multiple changes of media containing 0·75 m EDTA (Sigma, St Louis, USA) and 5% heat inactivated pooled human serum to remove epithelial cells. The tissue was then incubated overnight in Hanks's balanced salt solution collagenase medium containing 16 μ/ml chromatographically purified collagenase (Worthington Biochemical, Freehold, USA) and 20% heat inactivated pooled human serum. After collagenase digestion, cells were layered over a ficoll hypaque gradient (specific gravity (sg) 1·077) and centrifuged at 400 *g* for 20 minutes. The interface was collected, diluted with Hanks's balanced salt solution, and resuspended in 10 ml Percoll solution (sg 1·040, Pharmacia, Piscataway, USA), and centrifuged at 500 *g* for 15 minutes to remove dead cells and debris. These cells were then washed through fetal bovine serum gradients and counted.

ANTIBODIES

The CD45 common determinant marker (HLE-FITC), Leu 15-PE (CD11b), and the control antibodies IgG1-FITC, IgG-PE, IgG2a-FITC, and IgG2a-PE were purchased from Becton-Dickinson (Mountain View, California). B4-FITC, B4-RD1 (CD19), B1-FITC or B1-RD1 (CD20), and T1-RD1 (CD5) were purchased from Coulter (Hialeah, Florida). Biotinylated goat anti-mouse immunoglobulin and Tandem avidin were purchased from Southern Biotechnology (Birmingham, Alabama). UCHLI (CD45RO) was a generous gift of Dr P Beverley. Monoclonal antibodies to CD45RA were CD45RA FITC, purchased from GEN Track (Wayne, Pennsylvania) and FMC44-PE.[23 24]

The specificity of most CD45 antibodies used here was confirmed by their ability to precipitate bands having the appropriate molecular weights[25] (unpublished data) and all were tested for their reactivity with a panel of CD45 transfectants.[26]

THREE COLOUR IMMUNOFLUORESCENCE

Cell surface antigens present on the isolated mononuclear cells were evaluated by three colour immunofluorescence using a four stage combined direct and indirect staining procedure. In stage (a) cells were stained with an uncoupled antibody; (b) secondly with goat anti-mouse biotin (Jackson); (c) blocked with mouse Ig (Jackson), 1 μg/ml; and (d) stained with Streptavidin-Tandem, together with the two remaining antibodies directly conjugated to either FITC or PE. Mononuclear cells were resuspended in 50 μl of uncoupled monoclonal antibody diluted roughly in phosphate buffered saline containing 0·5% bovine serum albumin and 0·02% sodium azide. The cells were incubated for 30 minutes at 4°C, spun down, and washed twice in buffer solution, and incubated for 10 minutes at room temperature, spun down and resuspended in 20 μl Streptavidin-Tandem. The other two monoclonal antibodies coupled to

TABLE I *CD3+ Mucosal lymphocytes*

	CD45RA+ RO− (%)	*RA+ RO+* (%)	*RA− RO+* (%)	*RA−RO−* (%)
Crohn's disease (n=8)	23 (6)*	20 (6)	56 (9)	0
Ulcerative colitis (n=4)	22 (9)*	9 (5)*	57 (18)	12 (11)
Normal (n=10)	9 (2)*	19 (4)	66 (8)	6 (4)

CD45 isoform staining (CD45RA and RO) on CD3+ lamina propria lymphocyte T-cells as detected by three colour immunofluorescence. CD45RA was detected by FMC44PE. CD45RO was detected by UCHL1 and indirectly stained with biotinylated goat anti-mouse immunoglobulin followed by Tandem-avidin, and CD3 was detected by Leu4-FITC. Files of 20 000 cells were electronically gated to include only CD3+ cells and dot plots of CD45RA *v* CD45RO staining generated. The number of positive cells in each quadrant was determined in comparison with identically gated samples stained with Leu4-FITC, IgG1PE, and IgG2a biotinylated goat anti-mouse Ig/Tandem avidin. Values are reported as mean (SEM). *$p<0.05$ *v* normal; p value between all other pairs not significant.

FITC and PE were added directly and 25 µl of buffer added. This was incubated for 30 minutes at 4°C. Cells were washed three times and fixed with 1% formalin for flow cytometric analysis. Analysis of samples was performed on a FACScan (Becton-Dickinson). Dead cells and red cells were excluded by gating on forward angle light scatter and side scatter. All samples included staining with isotype matched control antibodies and unstained cells. List mode files were collected of 20 000 cells from each sample, and measurements of all three fluorochromes as well as forward and side scatter were recorded.

CALCULATIONS AND STATISTICAL METHODS

To determine percentages of CD3 and CD19 peripheral blood lymphocytes and lamina propria lymphocytes expressing a specific marker, flow cytometry was performed on these populations. Three colour immunofluorescence data were collected in list mode from the analysis of each sample of lymphocytes. Data were then gated on the T or B-cell population of interest (CD3 or CD19) and the CD45 isoform expression of these respective populations were plotted as histograms using a Becton-Dickinson FACScan workstation with FACScan software, in comparison with an identically gated population, stained with CD3 or CD19 and isotype control monoclonal antibodies.

Of those patients with Crohn's disease whose peripheral blood lymphocytes were analysed, clinical parameters including the erythrocyte sedimentation rate, disease duration, drugs, disease location, age of the patient, and disease severity (presence or absence of fistulas or extra-intestinal manifestations of disease) as well as the Crohn's disease activity index (CDAI) were obtained from chart review.[27] Multiple regression analysis was performed by SPSS/PC+ using these clinical variables with high or low molecular mass isoforms (CD45RA and/or CD45RO) CD45 and the combination of its isoforms as dependent variables. The Student's *t* test was used to compare CD45 isoform expression on T or B-cells between types of disease.

TABLE II *CD3+ Peripheral blood lymphocytes*

	CD45RA+ RO− (%)	*RA+ RO+* (%)	*RA− RO+* (%)	*RA−RO−* (%)
Crohn's disease (n=21)	34 (5)	10 (2)	37 (5)	16 (4)*
Ulcerative colitis (n=11)	44 (3)	19 (3)	37 (4)	0†
Coeliac disease (n=13)	41 (4)	9 (2)	44 (6)*	4 (3)*
Normal (n=7)	44 (6)	9 (3)	42 (3)	1 (0)*

Assessment of the CD45 isoform staining (CD45RA and RO) on CD3+ T-cells was detected by three colour immunofluorescence as described in Table I. Values are reported as mean (SEM).
*$p<0.05$ Crohn's disease *v* normal, Crohn's disease *v* UC, Crohn's disease *v* coeliac sprue; p value between all other pairs not significant.

Results

PREDOMINANT EXPRESSION OF CD45RO ON CD3+ T-CELLS OF MUCOSAL LAMINA PROPRIA LYMPHOCYTES FROM NORMAL, CROHN'S DISEASE, AND ULCERATIVE COLITIS PATIENTS

Lamina propria lymphocytes isolated from fresh intestinal mucosa were analysed from normal and diseased tissue. Normal mucosa was derived from patients with diverticular disease, areas distal to neoplasms, and deceased organ donors. Lymphocyte profiles were not significantly different between these groups of patients. Normal lamina propria lymphocytes were stained by three colour immunofluorescence with antibodies to CD3, CD45RA, and CD45RO. After gating for CD3+ T-cells, the proportion of each isoform (CD45RA and CD45RO) on CD3+ cells was measured (Table I). The patterns of CD 45 isoform expression on CD3+ T lamina propria lymphocytes from patients with Crohn's disease or UC was similar to those found among normal T lamina propria lymphocytes with a predominance of CD45RA− RO+ cells (mean (SEM) 66 (8)%, Table I) confirming previous published work.[2] Our ratio of CD45RA and CD45RO on normal and Crohn's disease lamina propria lymphocytes is consistent with published data. A confirmation of published works was necessary as a framework for comparing CD45 isoforms on B-cells to ensure our patients and normal controls did not vary from others.[2 17]

HETEROGENEITY OF CD45 ISOFORM EXPRESSION ON CD3+ T PERIPHERAL BLOOD LYMPHOCYTES FROM NORMAL, CROHN'S DISEASE, AND ULCERATIVE COLITIS PATIENTS

Normal PBMC were analysed, files were gated on CD3+, and CD45 isoform expression was assessed. No abnormalities in proportion of CD3, CD4 or CD8 cells were detected confirming previous reports.[28] Normal patients PBMC included 44 (6)% CD45RA+ RO− CD3+ cells, similar to that of UC (44 (3)%) or Crohn's disease (34 (4)%). No appreciable difference in CD45RA+ RO+ or CD45RO− RA+ lymphocytes were found between normal, Crohn's disease, and UC CD3+ PBMC (Table II). A detectable population lacking both CD45RA and RO was found among T-cells from Crohn's disease (16%, Table II) but not among those in normal subjects, UC, or coeliac sprue. Because all T-cells express CD45 common determinants, the possibility exists that this reflects either expression of an isoform not detected by our monoclonal antibodies, for example CD45RB p190, or a change in glycosylation. Further work is needed to establish its significance.

ANALYSIS OF CD45 ISOFORMS OF CD19+ B LAMINA PROPRIA LYMPHOCYTES FROM NORMAL, CROHN'S DISEASE, AND UC PATIENTS

CD19 lamina propria lymphocytes from IBD

TABLE III *CD19+ Mucosal lymphocytes*

CD45 (%)	*RA+ RO−* (%)	*RA+ RO+* (%)	*RA− RO+* (%)	*RA− RO−* (%)
Crohn's disease (n=6)	40 (8)	21 (7)	37 (10)	2 (1)
Ulcerative colitis (n=4)	45 (13)	26 (5)	29 (16)	0 (1)
Normal (n=10)	45 (5)	27 (4)	23 (6)	5 (2)

p Value between all pairs of data not significant.
CD45 isoform staining (CD45RA and RO) on CD19+ or CD20+ B-cell lamina propria lymphocytes as detected by three colour immunofluorescence. CD45RA was detected by FMC44PE. CD45RO was detected by UCHLI and stained indirectly with biotinylated goat anti-mouse immunoglobulin and Tandem-avidin, and CD19 by B4-FITC, or CD20 by B1-FITC. Similar results were obtained with either CD19 or CD20. Files were gated for CD19+ cells and CD45 isoform expression analysed as for Table I. The mean percentage of CD19+ B-cells in each disease studied were: normal blood donors mean=8 (0·5)%, Crohn's disease mean=10 (5)%, UC mean=8 (5)%, consistent with published data.[30] Values are reported as mean (SEM).

and from normal patients (organ donor, diverticulosis, and neoplasm resections) were analysed to determine expression of CD45RA and RO isoforms (Table III). A similar percentage of CD19+ B-lymphocytes (8–10%) was found among the conditions studied as well as among normal patients.

On gut lamina propria lymphocytes, a range of CD45 isoforms were identified on CD19+ B-cells. As a group, UC, Crohn's disease, and normal lamina propria lymphocytes CD19+ B-cells include more cells bearing a transitional pattern of CD45 isoform expression (RA+ RO+) or not bearing either isoform (RA− RO−) than are found among circulating B-cells (Table III). Among normal lamina propria lymphocytes the CD45RA+ RO− phenotype was detected on 45 (5)% of CD19+ B lymphocytes, identifying a mature resting B-cell population; 50–66% of B-cells in IBD and normal lamina propria lymphocytes expressed CD45RO, which appears on activated and late stage B-cells.[9 18] Expression of CD45RO was found on 23% of normal lamina propria lymphocytes B-cells, consistent with definition as a late stage B/pre-plasma cell. Coexpression of both CD45RA and CD45RO, consistent with definition as an activated B-cell, was found on 27% of normal lamina propria lymphocytes B-cells (Table III). Five per cent of normal CD19+ lamina propria lymphocytes lacked both CD45RA and RO.

INCREASED PROPORTION OF LATE STAGE CD45RO+ B-CELLS IN CD19+ B PBMC FROM CROHN'S DISEASE COMPARED WITH NORMAL DONORS OR COELIAC SPRUE AND UC PATIENTS

PBMC from Crohn's patients with detailed clinical descriptions were analysed in comparison with normal donors (Table IV). CD19+ B-cells from normal donor PBMC express almost exclusively CD45RA+ RO−, consistent with our previous work.[9 17] Similarly, B-cells from UC and coeliac sprue PBMC express this isoform almost exclusively (98 and 96% respectively). In contrast, PBMC from patients with Crohn's disease included 44% of B-cells with an abnormal phenotype, either lacking expression of CD45RA, or coexpressing CD45RA and CD45RO (Table IV). Fifteen per cent of CD19+ PBMC B-cells of patients with Crohn's disease expressed CD45RA− RO+, 13% coexpressed both CD45RA and RO, and 16% were CD45RA− RO−. Thus, these B-cells have a CD45 isoform distribution consistent with their definition as a population of late stage antigen activated B lymphocytes.

TABLE IV *CD19+ Peripheral blood mononuclear cells*

CD45 (%)	*RA+ RO−* (%)	*RA+ RO+* (%)	*RA− RO+* (%)	*RA− RO−* (%)
Crohn's disease (n=17)	58 (9)*	13 (4)†	15 (5)†	16 (5)†
Ulcerative colitis (n=11)	98 (0)*	2 (0)†	8 (0)†	0†
Coeliac (n=11)	96 (1)*	2 (1)†	1 (0)†	0†
Normal (n=9)	99 (1)*	0*	0*	0*

CD45 isoform staining (CD45RA and RO) on CD19+ B-cell peripheral blood mononuclear cells as detected by three colour immunofluorescence as described in Table III. Values are reported as mean (SEM).
*p<0·005 Crohn's disease *v* normal, Crohn's disease *v* UC, and Crohn's disease *v* coeliac sprue.
†p<0·05 Crohn's disease *v* UC and Crohn's disease *v* coeliac sprue.

The expression of CD45 isoforms on B-cells from Crohn's disease PBMC was very heterogeneous, unlike that from normal donors, UC or coeliac sprue. Figure 1 shows the consistently high and comparatively uniform CD45RA density on normal, UC, and coeliac sprue B-cells in contrast with the broad density distribution on PBMC B-cells from Crohn's disease. Crohn's B-cells included a clearly CD45RA negative population and a broad distribution of CD45RA+ cells. A similar degree of heterogeneity was evident for the CD45RO expression on B-cells (Fig 2). PBMC from Crohn's patients included a bimodal but heterogeneous population of CD45RO+ B-cells with predominantly high antigen density (Fig 2). In general, those cells with a low intensity of CD45RO were those cells coexpressing CD45RA (Fig 1 and Table IV).

CD19+ B PERIPHERAL BLOOD LYMPHOCYTES FROM NORMAL AND CROHN'S DISEASE PATIENTS ARE CD11b− AND MAINLY CD5−

CD5 and CD11b expression on CD19+ B-cell PBMC from patients with Crohn's disease was analysed. No CD11b was found on CD19+ B-cell PBMC from patients with Crohn's disease, or on B-cells from normal donors. A low level of CD5 expression (mean 7 (1)%, n=19) was found on CD19+ B peripheral blood lymphocytes in Crohn's disease patients, less than that found on B-cells from normal donors (mean=30 (5)%, n=7) (data not shown). As CD5 and CD11b have been shown to increase on the activation of B-cells the lack of either antigen on lamina propria lymphocytes B-cells argues against any activating effect of the procedures used to isolate lamina propria lymphocytes.[4 5 29]

CORRELATION BETWEEN THE PROPORTION OF CD45RO+ B-CELLS AND SEVERITY OF CROHN'S DISEASE

Considerable variability was detected in the expression of CD45RA or RO among CD19+ PBMC from individual patients with Crohn's disease, in clear contrast with the normal pattern (Fig 3). To assess the clinical significance of this considerable variation in CD45RA and RO expression on CD19+ B-cells, clinical data were obtained on each patient. A number of parameters known to be associated with Crohn's clinical disease severity were tabulated, and multiple regression analysis was performed on these values using the statistical program SPSS/PC+. The B-cell subsets defined by CD45

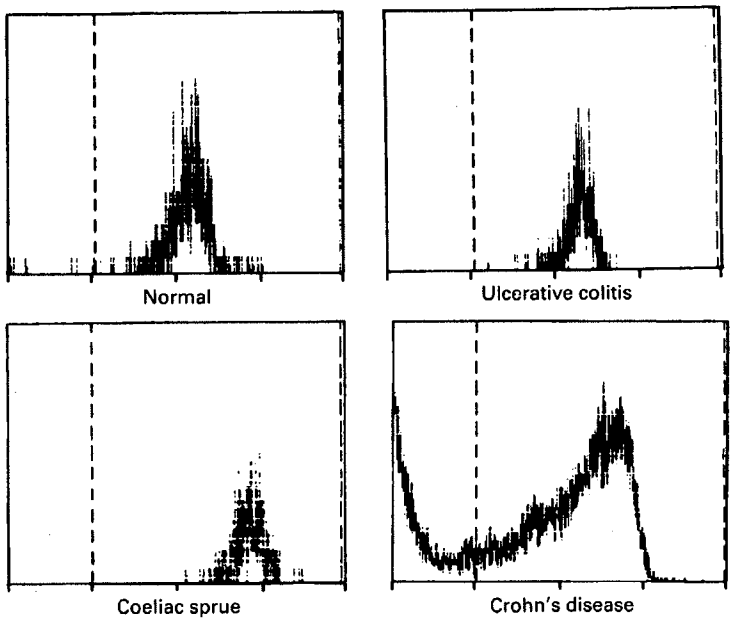

Figure 1: CD45RA expression on CD19+ PBMC B-cells. Normal, ulcerative colitis, and coeliac sprue FACScan histograms have consistently high and comparitively uniform expression of CD45RA in contrast with the heterogeneous distribution in peripheral blood mononuclear cells B-cells in Crohn's disease. Staining was as described in Table III. Files were gated for CD19+ cells and the distribution of CD45 isoform plotted. The vertical line denotes cell number and the horizontal axis the log fluorescence intensity. Cells were stained with CD19 or CD20 FITC, CD45RA PE, and CD45RO/biotinylated goat anti-mouse Ig/tandem-avidin. Files of 20 000 cells were collected, and then gated for CD19+/20+ cells followed by a plot of CD45RA staining. Similar results were obtained with either CD19 or CD20.

isoforms, as dependent variables, were compared with the clinical parameters; Crohn's disease activity index (CDAI), disease duration, erythrocyte sedimentation rate, drugs, age of patient, and disease severity, as independent variables. CD45RO+ B-cells were divided into two groups, group 1 included B-cells expressing 0–49% CD45RO+. Group 2 included all patients with B-cells having 50% and greater CD45RO+ expression. These values were compared with the respective CDAI values for each patient, showing the means of the two groups to be significantly different using the two sample *t* test (group 1 mean=108·2, SE=16·5) (group 2 mean=179·0, SE=27·4) (n_1=23, n_2=6, t=2·01, 2 tail p=0·05). Furthermore, there is a grouping of high values of CD45RA− RO+ with high CDAI values and vice versa. No other combination of parameters or any B-cell phenotype had such a significant relation. When CDAI was graphed against CD45RO, however, the relation was not linear, with some patients having high CDAI expressing low CD45RO on their CD19 cells and vice versa, suggesting that other parameters participate in the degree of expression of CD45RO on B-cells.

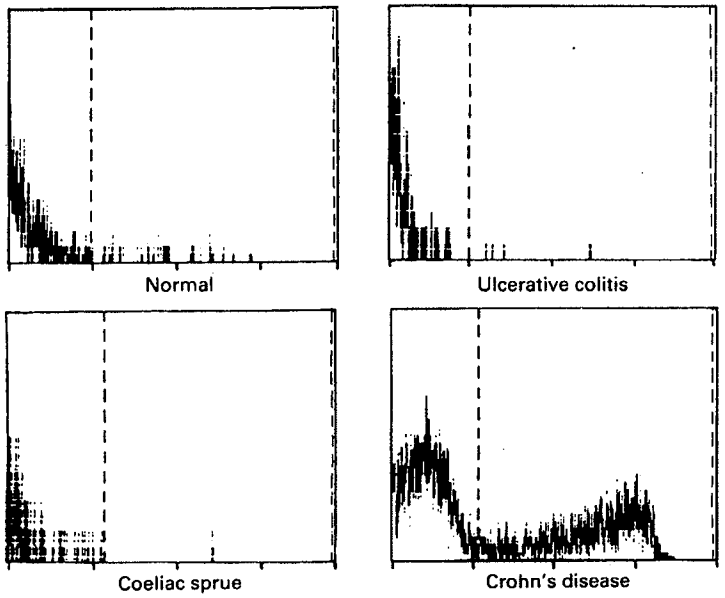

Figure 2: CD45RO expression on CD19+ PBMC B-cells. Files were gated for CD19+ or CD20+ cells and the expression of CD45RO plotted as a histogram. Negligible expression of CD45 RO+ is found on CD19+ B-cells from normal, coeliac sprue or ulcerative colitis patient peripheral blood. Peripheral blood mononuclear cells from Crohn's patients included a heterogeneous population of CD45RO+ B-cells with predominantly high antigen density. Similar results were obtained with either CD19 or CD20. Staining and analysis were as for Figure 1.

Discussion

In this report we have focused on the expression of CD45 and its isoforms RA and RO on PBMC or lamina propria lymphocytes, or both from a variety of intestinal diseases including Crohn's disease, UC, coeliac sprue, and normal control patients. Previous studies have been limited to immunohistochemical quantification of the CD45 isoforms on T-cells, or to heightened lymphocyte activation in peripheral blood not specific to Crohn's disease.[3 4 17 30]

Differences in immunoglobulin isotype production by lamina propria lymphocytes from patients with Crohn's disease compared with lamina propria lymphocytes from UC and normal controls has been shown by others, but this difference cannot be a result of cell activation alone because differentiated cells and not activated replicating cells are responsible for most of the immunoglobulin production.[4 21] Moreover, recognition of the expression of CD45RO on B-lymphocytes has been recent and until this report, primarily associated with some normal B-cells from aged adults, in vitro stimulated B-lymphocytes, and malignant B-cells.[9 18 19 24 31] Acquisition of CD45RO isoform on B-lineage cells occurs after in vitro stimulation[9 18] confirming the in vivo transition from CD45RA to CD45RO, a process accentuated in lamina propria lymphocytes and Crohn's disease PBMC. This study identifies a unique population of non-malignant CD45RO+ CD19+ PBMC that to date have been identified only after in vitro stimulation and in PBMC from patients with multiple myeloma, Waldenstrom's macroglobulinaemia, and chronic lymphocytic leukaemia.[31 32]

Our analysis of CD3+ lamina propria lymphocytes from the normal intestine confirms the prevalence of CD45RO as the predominant isoform (66 (8)%). Comparatively few antigen inexperienced (CD45RA+) or transitional forms (CD45RA+ RO+) were identified in normal lamina propria lymphocytes (9 (2)% and 19 (4)% respectively). These findings are consistent with previous published work.[1] This consistency with accepted published works was necessary to

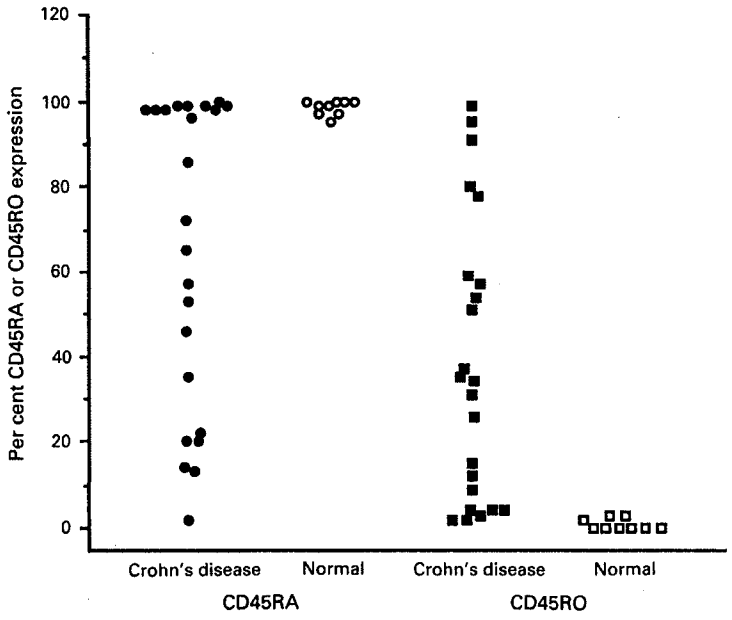

Figure 3: Percentage of CD19+ CD45RA+ or CD19+ CD45RO+ B-cells in Crohn's disease v normal peripheral blood mononuclear cells. Staining was as described in Figure 1.

provide a framework to compare CD45 isoforms on B-cells in these same patients.

B-cells from organ tissue (including spleen, lymph node, and thymus) also express CD45RA but unlike normal PBMC, these tissues also express a substantial proportion of CD45RO.[9] This may be as a result of their role as immunological responders to environmental antigens, given the presence of germinal centres in these tissues. Our data identify normal intestinal lamina propria lymphocytes as another tissue with a proportion of B-lymphocytes expressing CD45RO, probably because of the continuous antigen exposure of normal intestine. In contrast with the pattern of CD45 isoform expression among circulating B-cells in PBMC, equal expression of CD45RA and RO is found in normal CD19+ lamina propria lymphocytes. The pattern of CD45 isoforms on CD19+ lamina propria lymphocytes from inflamed, diseased IBD tissue closely resembled normal intestine, although some patients with Crohn's disease had increased numbers of CD45RO+ B-cells, however this difference was not statistically significant. CD19+ lamina propria lymphocytes B-cells from normal or diseased intestine have not been previously analysed specifically for CD45 isoform expression. The shift of B-cell CD45 isoforms, however, to a more terminally differentiated population in the gut mucosa is consistent with the heightened immune activation in gut found by others.[4 29] This comfirms that intestinal B-cells comprise an antigen stimulated subset at later stages of differentiation than seen for peripheral blood B-cells compatible with the activated state of normal intestine lamina propria lymphocytes.

CD45RA+ RO+ as well as RA− RO− subset phenotypes were represented among CD19+ PBMC from patients with Crohn's disease in contrast with UC or normal PBMC. This pattern of CD45RO+ and transitional isoform expression suggests a population of activated or late stage B-cells as might be predicted if they play a part in the general heightened immune response associated with Crohn's disease. This heterogeneity of CD45 expression on CD19+ PBMC from patients with Crohn's disease was statistically studied with a number of clinical parameters (including the CDAI, disease duration, extra-intestinal disease, disease location (colon, small bowel, colon and small bowel), erythrocyte sedimentation rate, drugs, and age of patient) by multiple regression analysis to determine if any of these variables could account for the aberrant pattern of CD45 isoform expression found on this population of PBMC. Of these clinical parameters, the CDAI was found to be statistically correlated with significance to the CD45RA− RO+ subpopulation of CD19+ PBMC in Crohn's disease. When CD45RO+ B-cell expression is graphed against the CDAI, however, the pattern obtained does not seem linear. This may be due to CD45RO expression being related to another variable such as changed intestinal permeability. Moreover, the CDAI is known to vary as a measurement of Crohn's disease severity and this also contributes to the dispersed appearance of the graph. Further studies of CD45RO in Crohn's peripheral blood lymphocytes are in progress and may show a more linear relation with other variables.

Although other explanations are possible, the most plausible suggests that the apparent correlation of CD45RA− RO+ CD19+ PBMC with the CDAI results from a stimulated immune system in more severely ill patients either as a primary event initiating intestinal inflammation or as part of an inflammatory cascade once inflammation has started. CD5 and CD11b were absent from lamina propria lymphocytes or PBMC B-cells suggesting these represent late stage B-cells rather than cells in the process of activation, which would express both antigens based on patterns of activation in vitro.[9] Recent work by MacDonald has shown an activated submucosal T-cell population in Crohn's disease patients intestine. Such a population could provide the necessary help to result in the high proportion of late stage and transitional B-cells found in Crohn's disease.[33] To our knowledge, no other population of differentiated, mature PBMC has been identified exclusively in patients with Crohn's disease. Drugs used could not explain the exclusive expression of CD45RO on B-cells in Crohn's disease because the same drugs were used in UC without CD45RO being found. Additionally, no statistical correlation with a specific drug could be identified to account for the presence of CD45RO+ B-cells.

Our finding of a trend towards an increased proportion of CD45RO+ and transitional lamina propria lymphocytes in Crohn's disease may reflect the increased accessibility of antigens to lamina propria lymphocytes in Crohn's disease because of the transmural inflammation characteristic of this illness. Alternately, the abnormal immune response identified in Crohn's disease may permit antigen penetration through mucosa resulting in increased lamina propria lymphocytes B-cell transition to the CD45RO isoform. The prevalence of the CD45RO isoform almost exclusively on CD19+ PBMC of patients with Crohn's disease thus may be due to a 'spill

over' of these transitional B-cells from gut into blood.

In conclusion, we have recorded the expression of CD45RO on circulating PBMC B-cells exclusive to IBD patients with Crohn's disease. The presence of high numbers of late stage CD45RO+ lymphocytes in the gut has been identified previously.[3 17] Our identification of CD45RO+ and transitional CD45 isoform B-cells in peripheral blood, however, defines a new cellular population of interest in IBD.

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Exhibit C

Immunology and Cell Biology (1993) **71**, 265–274

Activated CD19$^+$ B cell lamina propria lymphocytes in ulcerative colitis

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Summary Although isotype differences in lamina propria lymphocyte (LPL) immunoglobulin production has been recognized between normal, ulcerative colitis (UC) and Crohn's patients, differences in B cell activation between these conditions has not been described. Using flow cytometry, we studied B cell LPL activation using the CD71 (transferrin receptor), CD25 (interleukin-2 receptor) and 4F2 (early B and T cell activation marker) monoclonal antibodies. CD19$^+$ B cells from patients with UC had relatively increased expression of CD71, CD25 and 4F2 compared with patients with Crohn's disease or normal mucosa. This finding corresponds to an increased proportion of transitional (activated) B cells as defined by the co-expression of CD45RA and CD45RO found in UC LPL compared with normal or Crohn's patient LPL. Such data may identify an important link between the cellular state of activation of UC LPL B cells and the differences in immunoglobulin production between the inflammatory bowel diseases and normal intestine. Such findings may have further diagnostic or pathophysiologic importance in the study of these diseases. This work also provides further support for the CD45 transition of CD19 B cells from the high to low molecular weight isoform.

Key words: activation markers, CD45, flow cytometry, inflammatory bowel disease, lamina propria lymphocytes, monoclonal antibodies.

Introduction

The inflammatory bowel diseases (IBD), Crohn's and ulcerative colitis (UC), are systemic immunoregulatory disorders of unknown aetiology. Specific abnormalities of the mucosal immune system have been identified in IBD patients, but few studies have identified differences between UC and Crohn's disease. Discriminating between these diseases is important, since a large proportion of colitis is classified as indeterminate aetiology and differentiation is needed due to the different management and response to therapy for Crohn's disease and UC. For example, different patterns of immunoglobulin secretion (predominantly IgG) have been found with relatively increased IgG1 and IgG3 in UC and increased IgG2 in Crohn's disease.[1–4] Lamina propria lymphocytes (LPL) from patients with UC have a characteristic pattern of anti-neutrophil antibody production, suggesting that UC may also have an autoimmune component in the course of the disease.[5] Although to date the B cell populations producing these immunoglobulins have not been isolated, certain activation or maturation markers may identify them. For instance, in a mixed B and T cell isolation of LPL from patients with IBD, increased expression of some activation markers has been found.[6]

This study analysed the phenotypes of B cells from patients emphasizing the difference in expression of activation markers and the selective expression of CD45 isoforms, as a marker for differentiation within the B cell lineage, between Crohn's disease, UC and normal LPL. Monoclonal antibodies to surface

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antigens CD71 (transferrin receptor),[7–8] CD25 (interleukin-2 receptor) [9] and 4F2 (T and B cell early activation marker)[10–12] were used to identify differences in cellular activation between normal intestine and IBD diseased tissue.

The expression of the CD45 antigen was quantified on $CD19^+$ B cell LPL in UC, Crohn's disease and normal patients. CD45, also known as the leukocyte common antigen, is the most prevalent antigen on the surface of B and T lymphocytes and through its cytoplasmic domain, the tyrosine phosphatase activity plays a key role in intracellular signalling.[13] The CD45 isoforms p220 and p180 are distinguished by differences in molecular weight, glycosylations and are encoded by alternately spliced mRNA.[14–16] These isoforms characterize T cell differentiation with the transition from the expression of the high molecular weight CD45RA (p220) isoform on naive cells to the low molecular weight CD45RO (p180) isoform on memory T cells.[17–18] Recently, a study of $CD3^+$ intraepithelial lymphocytes from normal mucosa showed that this population expressed mainly CD45RO.[19] $CD3^+$ $CD45RO^+$ T cells are probable memory T cells consistent with their role as primary immune regulators in an antigen-bombarded environment such as the gut. However, B cells are also found among the mucosal lymphocytes in normal and diseased states.[6] B cells have recently been identified as having a similar transition in CD45 isoforms to that found in T cells.[20–22] Pre- B cells express exclusively CD45RA at low density with an increase in CD45RA density as differentiation proceeds towards mature B cell function.[20] A transition from CD45RA to CD45RO expression appears to occur on *in vivo* antigen-stimulated B cells which has been confirmed by *in vitro* studies.[21,22] Early plasma cells express only the low molecular weight CD45 isoforms, while end stage plasma cells eventually lose all CD45 expression.[22] Such CD45 transitions can be somewhat approximated by pokeweed mitogen stimulation of B cells *in vitro*.[22]

In this study, LPL $CD19^+$ B cells have been characterized in UC, Crohn's disease and normal patients by a number of cellular activation and maturation surface antigens. From the observations in this study, LPL from patients with UC contain a unique population of activated $CD19^+$ B lymphocytes.

Materials and methods

Patients and controls

Specimens of colon from patients with Crohn's disease and UC were obtained with the assistance of a pathologist to sample disease-involved mucosa. Normal mucosa was obtained from the colon at least 15 cm distal to carcinoma in patients undergoing resection as well as from normal areas in patients having resection for diverticulosis. Normal colon was also obtained from deceased persons donating organs for transplantation. A total of six 'normal', 11 Crohn's disease and five UC colons were analysed. Specimens were not analysed from UC patients with fulminant colitis nor from organ donors with longer than 48 h of hospitalization prior to organ harvest.

Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMC) were obtained with informed consent from seven normal Red Cross blood donors. PBMC were purified by a 30 min 400 *g* centrifugation at room temperature over Ficoll Paque (Pharmacia, PQ, Canada) followed by two washes in Hank's Balanced Salt Solution (HBSS).

Isolation of lamina propria lymphocytes

Normal large bowel intestinal mononuclear cells (INT MNC) were obtained from surgically removed specimens from individuals donating organs for transplantation from patients with diverticulosis as well as from morphologically normal areas of large bowel at least 15 cm distal to diseased areas of colon resected for adenocarcinoma. Crohn's disease and UC INT MNC were obtained from colon specimens from individuals having resections done for therapeutic reasons. Intestinal MNC were isolated from mucosa according to a well established protocol as follows.[4,23,24] Briefly, specimens were washed in RPMI media and the mucosa dissected from the submucosa.

The mucosa was then cut into small 0.5 cm × 0.5 cm minced pieces and washed repetitively in HBSS without calcium and magnesium containing 1 mg/mL Ticarcillin (Beecham, Pointe-Claire, QC, Canada), 0.5 mg/mL Amikacin (Bristol Labs, Ottawa, ON, Canada), 0.4% (w/v) Septra (Burroughs-Wellcome, Kirkland, QC, Canada), 2 mg/mL Fungizone Grand Island Biological Co. (Gibco, Grand Island, NY, USA), 10 mmol/L HEPES and NaOH to adjust to pH 7.4. The minced pieces were stirred in multiple changes of media containing 0.75 mol/L ethylenediamine tetra-acetic acid (Sigma, MO, USA) and 5% (v/v) heat-inactivated pooled human serum to remove epithelial cells. The tissue was then incubated overnight at 37°C in HBSS–collagenase medium containing 16 U/mL chromatographically purified collagenase (Worthington Biochemical, Freehold, NJ, USA) and 20% (v/v) heat-inactivated pooled human serum. Following collagenase digestion, cells were layered over a Ficoll-hypaque gradient (specific gravity 1.077) and centrifuged at 400 *g* for 30 min at room temperature. The interface was collected, diluted with HBSS and resuspended in 10 mL Percoll solution (specific gravity 1.040; Pharmacia, Piscataway, NJ, USA) and centrifuged at 500 *g* for 15 min to remove dead cells and debris. These cells were then washed through fetal bovine serum gradients and counted.

PBMC were isolated from the heparinized blood of normal healthy volunteers using Ficoll hypaque centrifugation.[3] Isolated cells were stimulated with pokeweed mitogen (Gibco Laboratories) at a final dilution of 1 : 100 in RPMI-1640 with 10% heat-inactivated fetal calf serum in 1 g glass vials in a 5% CO_2 humidified atmosphere at 37°C for 72 h, stained with monoclonal antibodies (staining procedure as described for three colour immunofluorescence) and analysed by fluorescence-activated flow cytometry.

Antibodies

The CD45 marker (HLE-fluorescien isothyocyanate [FITC]), CD25(Il-2R), CD8, CD4 and control antibodies, IgG1-FITC, IgG, phycoerythrin (PE), IgG2a, FITC and IgG2aPE, were purchased from Becton-Dickinson (Mountain View, CA, USA). B4-FITC, B4-RD1 (CD19), B1-FITC or B1-RD1 (CD20) were purchased from Coulter (Hialeah, FL, USA). Biotinylated goat antimouse immunoglobulin and Tandem avidin were purchased from Southern Biotechnology (Birmingham, AL, USA). UCHL1 (CD45RO) was a generous gift from Dr P. Beverley (University College, London, UK). Monoclonal antibodies to CD45RA were CD45RA FITC, purchased from GEN Track (Wayne, PA, USA). 5E9 (CD71) and 4F2 clones were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA).

The specificity of most CD45 antibodies used here was confirmed by their ability to precipitate bands having the appropriate molecular weights,[25] and all were tested for their reactivity with a panel of CD45 transfectants.[26]

Three colour immunofluorescence

Cell surface antigens present on the isolated MNC were evaluated by three colour immunofluorescence (IF) using a four stage combined direct and indirect staining procedure: (i) cells were stained with an uncoupled antibody; (ii) secondly with goat anti-mouse biotin (Jackson); (iii) blocked with 1 μg/mL mouse Ig (Jackson); and (iv) stained with Streptavidin-Tandem, together with the two remaining antibodies directly conjugated to either FITC or PE. MNC were resuspended in 50 μL of uncoupled monoclonal antibody diluted approximately in phosphate-buffered saline containing 0.5% bovine serum albumin and 0.02% sodium azide. The cells were incubated for 30 min at 4°C, centrifuged at 400 *g* for 10 min and washed twice in buffer solution and incubated for 10 min at room temperature, centrifuged and resuspended in 20 μL Streptavidin–Tandem. The other two monoclonal antibodies coupled to FITC and PE were added directly and 25 μL of buffer added. This was incubated for 30 min at 4°C. Cells were washed three times and fixed with 1% paraformaldehyde for flow cytometric analysis. Cell surface staining was performed using the following monoclonal antibodies: 5E9 (which recognizes the human transferrin receptor CD71, clone 01.1; ATCC), 4F2

(recognizing an early activation antigen on T and B cells; ATCC), CD25 (IL-2R), CD8 (cytotoxic/suppressor T cell), CD22 (mature B, clone S-HCL-1), CD19 (Pan B, clone 4G7) and CD4 (T helper cell, clone SK3) all from Becton Dickinson (CA, USA). Analysis of samples was performed on a FACScan (Becton Dickinson). Dead cells and red cells were excluded by gating on forward angle light scatter and side scatter. All samples included staining with isotype-matched control antibodies and unstained cells. List mode files were collected from 20 000 cells from each sample, and measurements of all three fluorochromes as well as forward and side scatter were recorded.

Calculations and statistical methods

To determine the percentages of CD3 and CD19 LPL expressing a specific marker, flow cytometry was performed. Three-colour IF data were collected in list mode from the analysis of each sample of lymphocytes. Data were then gated on the T or B cell population of interest (CD3 or CD19), and the CD45 isoform or activation marker antibody of interest were plotted as histograms using a Becton-Dickinson FACScan workstation and FACScan software. Statistical analysis was performed using Student's *t*-test.

Results

Expression of CD71 on CD19$^+$ LPL B cells from UC, Crohn's disease and control patients

LPL from IBD and normal patients were analysed to determine the expression of CD71 on CD19$^+$ B cells. In all conditions studied, 8–10% of the LPL were CD19$^+$ B cells. Although the percentage of CD19$^+$ LPL that co-expressed the CD71 surface antigen was different, both the CD19$^+$ CD71$^+$ LPL from normal and Crohn's disease were low (10 and 13% respectively; Fig. 1). However, the CD19$^+$ CD71$^+$ LPL from patients with UC was significantly elevated. Thirty-five per cent of these CD19$^+$ cells expressed CD71. This increased percentage of CD19$^+$ LPL B cells from patients with UC is similar to levels of expression found on 72 h pokeweed mitogen-stimulated peripheral blood lymphocytes (PBL; Fig. 1). The expression of CD71 on normal and Crohn's CD19$^+$ LPL is similar to that of unstimulated PBL, suggesting that the LPL activation is not secondary to the LPL isolation procedure (Fig. 2).[27] The CD19 cells were also analysed for the expression of surface antigens recognized by both the 4F2 and CD25 monoclonal antibodies. Our results demonstrate that these activation markers are increased on UC CD19$^+$ cells over the expression found on normal INT MNC, which is consistent with previous reports (Fig. 3).[6] Normal intestinal LPL B cells maintain a low but detectable degree of activation, but this is much less than for UC LPL B cells (Fig. 4). No association was identified between the age of the patients studied, medications used or the sex of the patient and the expression of CD71 on CD19 B cells.

Expression of CD45 isoforms on CD19$^+$ B LPL from UC, normal and Crohn's disease patients

CD19$^+$ B cells in LPL isolated from patients with Crohn's disease, normal and UC patients express a range of CD45 isoforms. Although we have looked at a few patients, it appears that a pattern for each can be distinguished (Table 1). Normal LPL CD19$^+$ B cells typically express the CD45RA isoform, but transitional (CD45RA$^+$RO$^+$) and differentiated (CD45RO) antigens are found as well. In Crohn's disease, CD19$^+$ LPL B cells of more differentiated CD45 isoforms are characteristic and this shift of isoforms in the gut mucosa is consistent with the increased activation observed in the gut. UC LPL CD19$^+$ B cells expressed a pattern of CD45 isoforms different from that of normal and Crohn's disease patients (Fig. 5). Forty-four per cent of UC LPL CD19$^+$ B cells were CD45RA$^+$RO$^-$, which is consistent with their definition as a resting population. Thirty-six per cent were CD45RA$^+$RO$^+$, a transitional stage, while the proportion of late stage CD45RA$^-$RO$^+$ or CD45RA$^-$RO$^-$ was variable between UC patients.

Significantly more B cells from UC LPL expressed CD45RA$^+$RO$^+$ than Crohn's mucosa. UC CD19$^+$ cells appear to express a

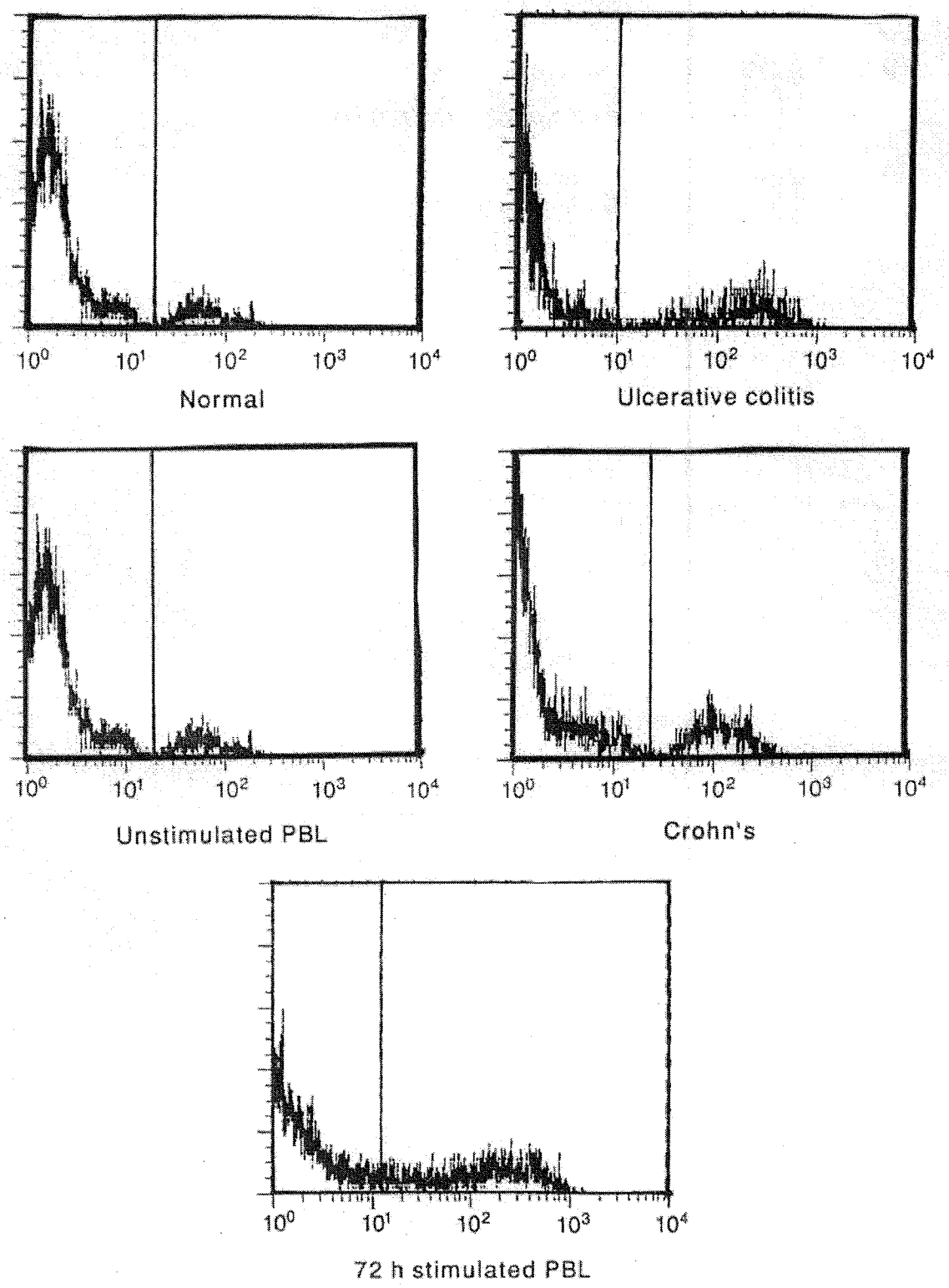

Fig. 1. CD71 expression on CD19⁺ lamina propria lymphocytes. FACScan histograms of representative LPL samples. Files were gated to include only CD19⁺ B cells and the expression of CD71 was plotted. Isotype-matched control samples were also identically gated and the control staining on B cells plotted and recorded as a solid line. 72 h pokeweed mitogen-stimulated PBL CD19⁺ B cells expressed similar amounts of CD71 as UC LPL CD19 B cells. Histograms were plotted with the Y axis indicating the relative cell number. The X axis represents fluorescence intensity.

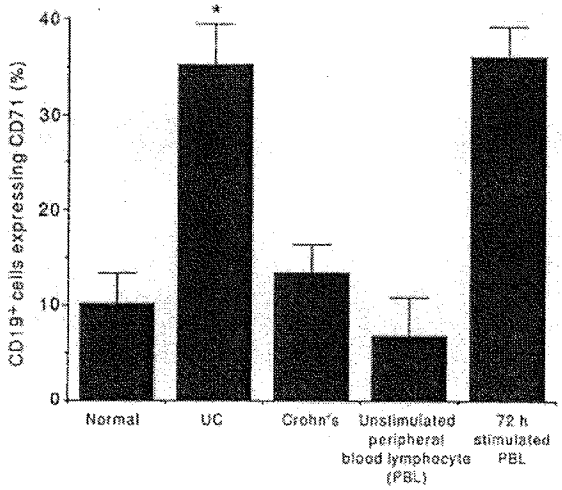

Fig. 2 Expression of CD71 on $CD19^+$ lamina propria B lymphocytes. The expression of CD71 on $CD19^+$ LPL cells from normal ($n = 7$), UC ($n = 12$) and Crohn's disease ($n = 7$) was analysed. Controls for CD71 expression included unstimulated and pokeweed mitogen-stimulated ($n = 3$) peripheral blood lymphocytes (PBL) $CD19^+$ cells. Values stated are % mean ± s.e.m.

transitional isoform of the CD45R antigen, demonstrating a difference in the status of the LPL of UC patients compared with normal and Crohn's disease patients. Furthermore, the increased proportion of transitional B cells, as identified by their expression of CD45RO, is consistent with the increased expression of CD71, CD25 and 4F2 activation surface antigens.

Discussion

The clinical presentation of UC is characterized by a systemic derangement of immune function. For example, UC may be associated with arthritis, uveitis, erythema nodosum and antineutrophil antibody production.[5] Some groups studying IBD lamina propria lymphocytes have identified the presence of autoantibodies as indirect evidence of abnormal B cell populations, suggesting that immunoglobulins may play a role in the pathogenesis of IBD as well as characterizing autoimmune diseases.[5,28] For example, IgG1 specific to surface antigens on colonic epithelium has been identified as deposits co-localized with activated complement and terminal complement complexes in UC patients.[29] Extraction of tissue-bound IgG from the UC specimens had shown reactivity to be specific to the 40 kDa colon-specific epithelial membrane glycoprotein.[28,30] Other groups have identified lymphocytotoxic antibodies that react to a wide variety of HLA-DR antigens as well as antigens found on neutrophils.[31,32]

In this study of UC, Crohn's disease and normal mucosa, we present evidence of an unrecognized difference in the $CD19^+$ LPL cells of IBD patients. Specifically, we demonstrate a difference in the expression of particular activation antigens as well as CD45 isoforms between UC, Crohn's disease and normal $CD19^+$ LPL cells. $CD19^+$ B cells from tissues where environmental antigens are presented in the germinal centres (i.e. spleen, lymph nodes and thymus) are know to express both CD45RO and CD45RA.[21] Coexpression of both these isoforms indicates cellular activation and transition that progresses with the loss of CD45RA and the acquisition of high density CD45RO.[20] Our data demonstrate the co-expression of $CD45RA^+$ and $CD45RO^+$, which has only recently been recognized.[22] Patients with UC have increased numbers of $CD45RA^+RO^+$ B cells in LPL relative to normal LPL. In control patients, $CD19^+$ B cell LPL express relatively more CD45RA, while Crohn's disease patient's $CD19^+$ cells express a greater percentage of $CD45RA^-RO^+$. The collection of the $CD19^+$ LPL cells in this transitional state suggests that these cells have somehow been activated but have not been able to progress due to a lack of the appropriate signalling, due either to a lack of exogenous lymphokines or the internal failure of appropriate signals.

Further evidence of the activated and transitional state is the increased expression of CD71, CD25 and 4F2, which are found on the $CD19^+$ cells of UC but not in normal or Crohn's disease specimens. The patients studied were relatively closely matched for age, sex and medications, with no statistical correlation attributable to these variables. The expression of CD71 found on the UC INT MNC B cells was the same as 48 h pokeweed mitogen-stimulated $CD19^+$ peripheral blood lymphocytes (PBL). Interestingly, the percentage of $CD19^+$ cells expressing

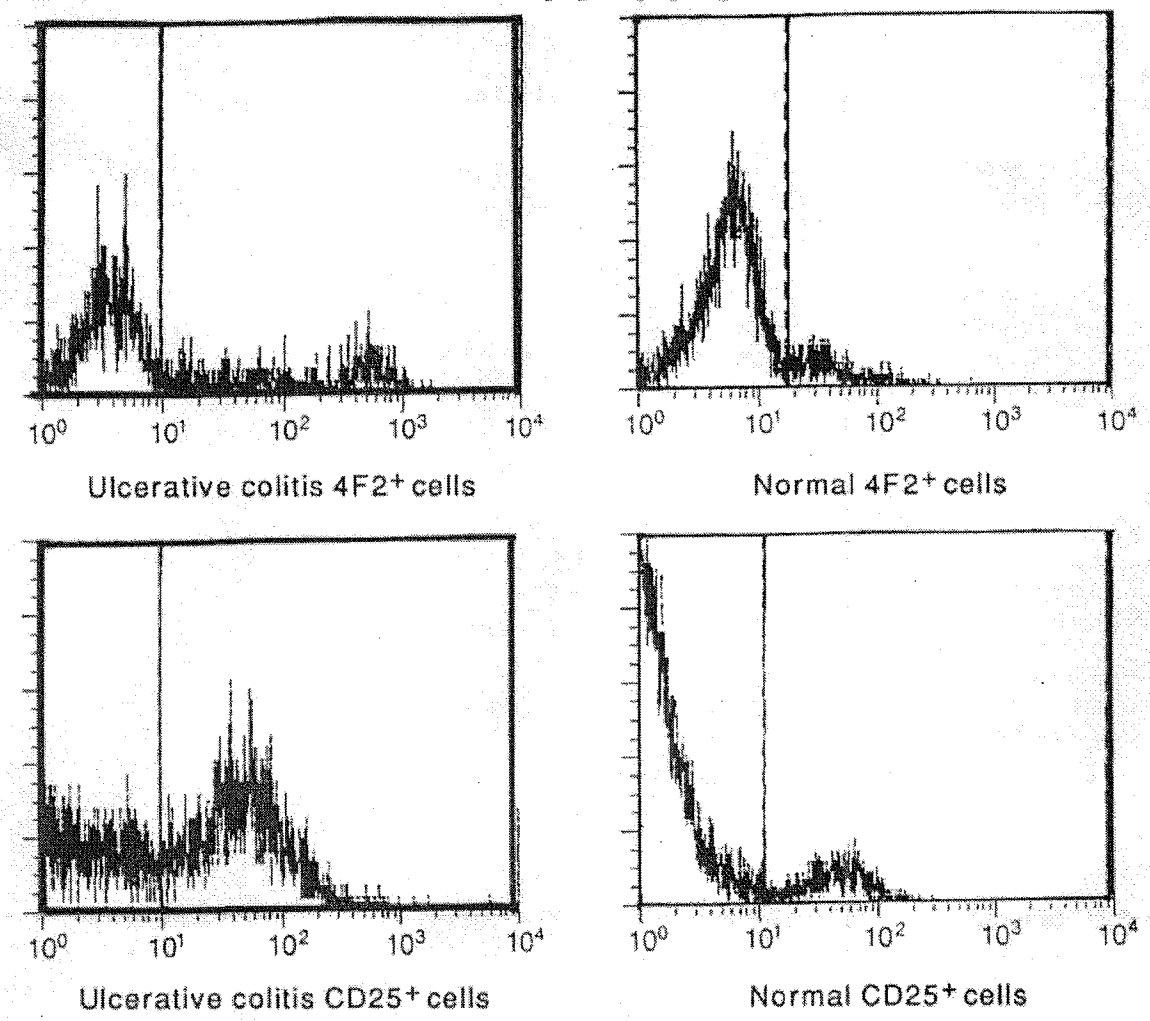

Fig. 3. 4F2 and CD25 expression on CD19⁺ LPL from normal and UC intestine. Representative FACScan histograms were gated to include only CD19⁺ B cells, and the expressions of 4F2 and CD25 were plotted. Isotype-matched control samples were also identically gated and the control staining on B cells plotted and recorded as a solid line. Histograms were plotted with the Y axis indicating the relative cell number. The X axis represents fluorescence intensity.

$CD45RA^+RO^+$ is similar to the relative percentage expression of CD71 (36 and 35%, respectively). Similarly, in Crohn's disease, 13% of the $CD19^+$ cells express CD71 and 11% express the $CD45RA^+RO^+$ pattern, suggesting that the $CD19^+$ $CD45RA^+RO^+$ are activated while being in a transitional state. Although our results are consistent with Schrieber *et al.* (1991)[6] with regard to an increase in the pattern of $CD19^+$ cell activation, they did not find that there was a different pattern between UC and Crohn's disease. This discrepancy may be attributable to the high degree of autofluorescence noted in their results, which was not a problem in the present study.

Although both IBD and normal mucosa express a range of CD45 isoforms, our data show that Crohn's $CD19^+$ cells bear more $CD45RA^-RO^+$ than UC or normal specimens. This finding is consistent with the higher expression of activated $CD25^+$ T cells found in Crohn's disease submucosa but not in UC submucosa.[33] This activated population of T cells not identified in UC LPL may provide greater exogenous lymphokine signals

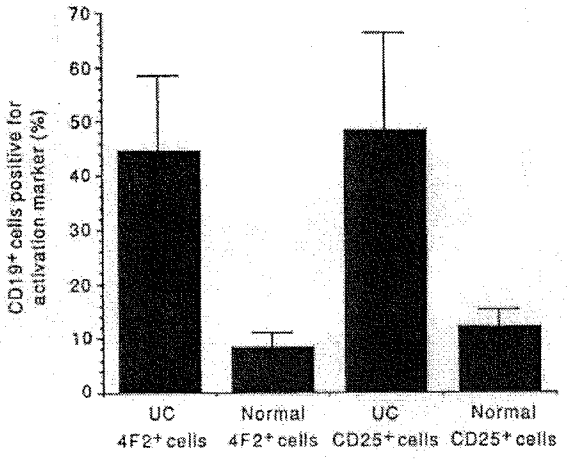

Fig. 4 Expression of 4F2 and CD25 on $CD19^+$ LPL cells. UC and normal $CD19^+$ B cell LPL comparative CD25 and 4F2 activation. Both CD25 expression (IL-2 receptor) and 4F2 (early activation antigen) are more prevalent in UC LPL than in normal LPL. Values stated are % mean ± s.e.m. The expression of 4F2 and CD25 cells was analysed using $CD19^+$ LPL cells from UC ($n = 7$) and normal ($n = 3$) samples.

necessary to move the $CD19^+$ cells from resting $CD45RA^+RO^-$ through to mature $CD45RA^-RO^+$, while in UC the T cells are not as activated (at least in regard to CD25), and as a result more $CD19^+$ cells collect in the transitional and activated state. Therefore, clinically our data would be consistent with the hypothesis that Crohn's disease is primarily a T cell mediated illness.[34] James (1988) has published clinical data supporting this, demonstrating that, with the acquisition of the human immunodeficiency virus, Crohn's disease appears to improve whereas UC does not.[34]

Although our overall results for both UC and Crohn's disease suggest that intimate antigen exposure through ulcerated mucosa leads to ongoing B cell activation, it appears that for UC a greater percentage of the $CD19^+$ LPL are in a transitional state, as seen by an increased expression of $CD45RA^+RO^+$ $CD19^+$ cells. Moreover, the CD19 LPL cells found in the Crohn's disease mucosa also express $CD45RA^+RO^+$; however more $CD19^+$ LPL cells express the $CD45RA^-RO^+$ phenotype, suggesting that the cells in Crohn's disease have further differentiated and may function differently to those CD19 LPL in UC. The significance of these objective immunological differences between Crohn's disease and UC is important given the diagnosis of indeterminate colitis and the impact that correct diagnosis imposes on the patient. These finds are compatible with the proposed T cell-mediated basis for Crohn's disease and a polyclonally activated B cell population in UC producing a range of autoantibodies.

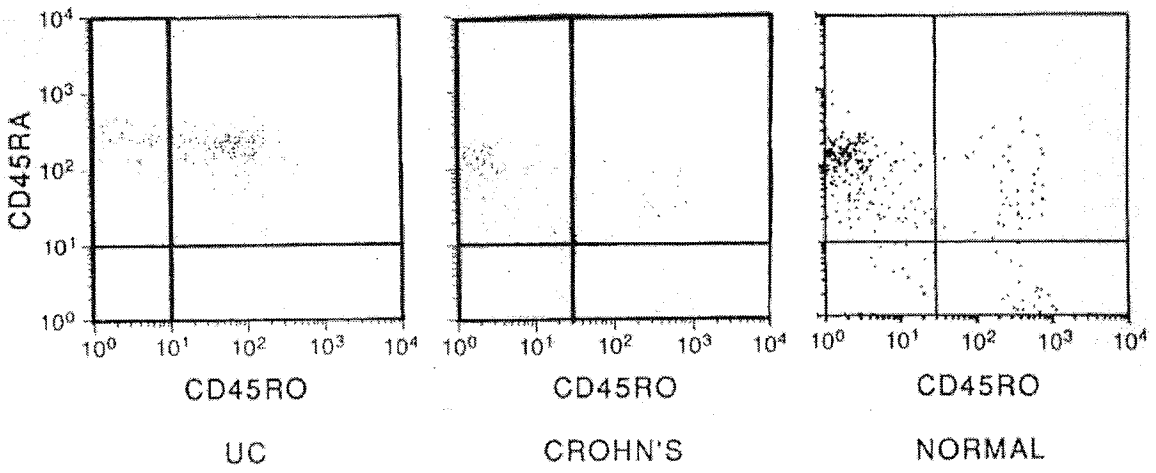

Fig. 5. Typical dot plot analyses of $CD19^+$ LPL B cells from a patient with Crohn's disease, UC and a normal deceased organ donor control. $CD19^+$ cells were analysed by three colour immunofluorescence by CD45 isoform staining (CD45RA and CD45RO). Crohn's disease ($n = 11$), UC ($n = 5$), normal ($n = 6$).

Table 1. CD45 isoform staining (CD45RA and RO) on $CD19^+$ or $CD20^+$ B-cell lamina propria lymphocytes as detected by three colour immunofluorescence (similar results were obtained using either CD19 or CD20

CD45	RA^+RO^-	RA^+RO^+	RA^-RO^+	RA^-RO^-
Crohn's ($n = 11$)	59 ± 9	11 ± 3	24 ± 8	5 ± 2
UC ($n = 5$)	44 ± 10	36 ± 6	17 ± 7	2 ± 1
Normal ($n = 6$)	61 ± 4	20 ± 4	14 ± 3	5 ± 3

The mean percentage of $CD19^+$ B-cells in each disease studied were: normal blood donors mean = $8 \pm 0.5\%$, Crohn's disease mean = $10 \pm 5\%$, UC mean = $8 \pm 5\%$, consistent with published data.[30] Values are reported as mean percentage ± s.e.m. percentage. Septic patients and patients with toxic or fulminant colitis were not included.

Acknowledgements

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Exhibit D

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ARTICLE

Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes

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To elucidate the biological dysregulation underlying two forms of inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD), we examined global gene expression profiles of inflamed colonic tissue using DNA microarrays. Our results identified several genes with altered expression not previously linked to IBD. In addition to the expected upregulation of various cytokine and chemokine genes, novel immune function-related genes such as *IGHG3*, *IGLL2* and *CD74*, inflammation-related lipocalins *HNL* and *NGAL*, and proliferation-related *GRO* genes were over-expressed in UC. Certain cancer-related genes such as *DD96*, *DRAL* and *MXI1* were differentially expressed only in UC. Other genes over-expressed in both UC and CD included the *REG* gene family and the calcium-binding S100 protein genes *S100A9* and *S100P*. The natural antimicrobial defensin *DEFA5* and *DEFA6* genes were particularly over-expressed in CD. Overall, significant differences in the expression profiles of 170 genes identified UC and CD as distinct molecular entities. The genomic map locations of the dysregulated genes may identify novel candidates for UC and CD genetic susceptibility.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD), two common inflammatory bowel diseases (IBDs) with shared clinical and demographic characteristics, harbor key differences in tissue damage and prognosis that suggest distinctive etiopathogenic processes. In UC, inflammation with crypt abscess formation is limited to the mucosa, while in CD, transmural granulomatous inflammation leads to fibrostenotic lesions and fistula formation (1). Both are complex clinical entities in which genetic, environmental and microbial factors interact to determine the susceptibility response of immune and non-immune cellular systems mediating inflammation (2,3). Mapping studies suggest a strong inherited component but a large number of putative susceptibility loci have complicated the identification of IBD genes. Confirmed IBD susceptibility regions include 16p12–q13 (IBD1), 12p13.2–q24.1 (IBD2), the major histocompatability complex region on chromosome 6 (IBD3) and 14q11–12 (IBD4) (4–9). Regions awaiting confirmation have been identified on 1p36, 3p21.2, 3q, 4q, 5q, 7q, 14q11–12 and 19p13 (5,10,11).

So far, immunoglobulins, eicosanoids, selected cytokines and immune activation gene products have been primarily studied with no definitive findings (12–14). As a complementary approach to genome-wide searches for IBD genes and to narrow down candidate gene searches, we investigated global gene expression patterns in UC, CD and normal control colonic tissue using DNA microarrays. The results show that the expression profiles of UC and CD are quite different and establish each form of IBD as a distinct molecular entity.

RESULTS

Microarray reproducibility

To assess array- and hybridization-based experimental variability, we initially performed hybridization on duplicate microarrays using the same pool of target RNA (Fig. 1, UC set 1 in experiments 1 and 2). Using all 7306 genes and expressed sequence tags (ESTs) as data points, a linear regression analysis was performed on the average fluorescence intensity difference per gene obtained in experiments 1 and 2. The two experiments were highly reproducible: 99.8% of the genes showed <3-fold difference in response, with a correlation coefficient of 0.97 between the two experiments. Only 12 out of 7306 genes and ESTs showed a >3-fold difference, all due to experimental error or chance. Therefore, for comparative profiling of UC versus control and CD versus control, we

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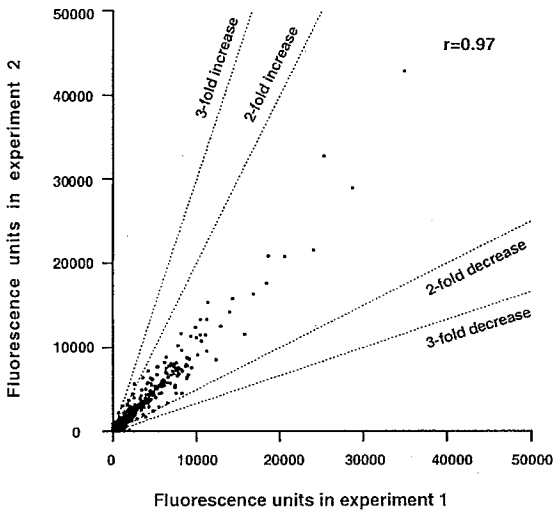

Figure 1. Reproducibility of microarray assays. Fluorescence intensity difference between perfect match oligonucleotides and mismatch oligonucleotides for each gene in experiment 1 (x-axis) and a duplicate experiment 2 (y-axis) were plotted to assess chance variation. Among 7306 transcripts studied in common, only 50 genes and ESTs showed ≥2-fold difference in expression, while at the ≥3-fold level, the response of 12 genes/ESTs was different, corresponding to $P = 0.0068$ and 0.0016, respectively. The correlation coefficient between the duplicate experiments was 0.97, so that only 3% of all the variations observed were due to experimental error. This high reproducibility between duplicate hybridization was used as an objective criterion in later experiments to assess which expression levels in the UC and CD samples were significantly different from those observed in controls. We chose the more stringent 3-fold threshold because at this level, in the reproducibility experiment, 12 out of 7306 genes and ESTs were false positives. Therefore, among the 170 differentially regulated genes in UC and CD profiles (Fig. 4) the expected number of false positives is 0.28 (12/7306 × 170). At the 2-fold level on average at least one of the genes identified would have been a false positive (50/7306 × 170 = 1.16).

defined a 3-fold change as the significance threshold to claim differential expression (Fig. 1).

RT–PCR validation of selected transcripts

A pool of RNA from colonic tissue samples of six UC, six CD and six control subjects was used for gene expression profiling. All CD and UC samples displayed a similar degree of histological inflammation and derived from patients with clinically comparable degrees of disease activity.

Prior to sample pooling, patient-to-patient variability in selected transcript levels (*MMP1*, *COL1A1*, *COL3A1*) was explored by semi-quantitative RT–PCR. The results of control, UC and CD ($n = 10$ in each group) for *COL1A1*, *COL3A1*, *MMP-1*, *MMP-3*, *MMP-12*, *TIMP-1*, *elafin* and *SPARC* transcripts are shown in Figure 2. Although some expected variability among individuals was observed, transcript levels for the genes tested were generally similar. In UC, transcripts for all the genes tested were significantly higher than the control ($P < 0.05$), in agreement with the results of the subsequent microarrays. For *TIMP-1*, although the RT–PCR results showed elevated transcripts, the UC profile did not detect them because the oligonucleotide probes initially used in the arrays were primarily of intronic origin (GeneChip Expression Analysis Sequence Information Database). RT–PCR of CD RNA showed elevated expression of *COL1A1* and *MMP-1* but no change in *SPARC*, *MMP-3* or *COL3A1* expression, also in agreement with the microarray results.

Variability in gene expression profiles of two independent sets of UC RNA samples

To identify changes in gene expression patterns in IBD compared with control tissue we hybridized biotinylated cRNA prepared from pooled poly(A)$^+$ mRNA to high density oligonucleotide microarrays (HuGene FI arrays 900160 and 900183). The absolute analysis (GeneChip Expression Analysis software, Affymetrix) yielded an average fluorescence difference for each gene and a call of absent, present or marginally present for each transcript level of the target sample (UC, CD and control). The comparison analysis was performed to determine the relative change in abundance for each transcript between a baseline (control) and an experimental sample (UC or CD) (the complete data may be viewed at http://www.jhmi.edu/~schakrav). To estimate variability between independent experiments, two different UC RNA sets (UC 1 and UC 2, six patients comprising each) were profiled. The same control RNA (also a pool of six individual samples) was profiled each time to generate baseline control 1 and 2 for the UC 1 and UC 2 experiments, respectively. Compared with control set 1, 140 genes were expressed differentially at the >3-fold level in UC 1. Figure 3 shows that expression of these 140 transcripts was similarly regulated in the second profiling with UC 2. Of the 10 most over-expressed transcripts in UC 1, seven were also the most over-expressed in UC 2, whereas 8 of the 10 most repressed genes in UC 1 were also repressed in UC 2. When the fold change in gene expression in UC 1/control 1 and UC 2/control 2 were compared by linear regression analysis, a positive correlation ($r = 0.65$) was detected, indicating a consistent pattern in gene expression in the two independent UC groups compared with the control group.

Next, we analyzed the gene expression pattern in UC 1, CD and control 1, comparing each IBD profile to the control 1 profile (Table 1 and Fig. 4). Numbers in the 'Increase' and 'Decrease' columns represent fold increase and decrease, respectively, in IBD gene expression over control. A total of 170 genes were differentially expressed in UC and CD (Table 1). A large number of them have not been previously associated with IBD, such as small inducible cytokine genes, *SCYA2* and *SCYA4*, *Annexin A5*, metallothionein genes, *MT1H*, *MT1G*, *S100A11*, *Elafin*, *SPARC*, collagen genes, *COL6A3*, *COL6A2* and von Willbrand factor gene, *vWF*, to name a few. We also found differential expression of genes for known inflammation-associated proteins such as HLA class II antigens, immunoglobulins, chemokine superfamily members, interleukin-1β, serum amyloid A protein and phospholipase A2, providing biological validity to the results of the microarray analysis.

Chromosomal locations of differentially expressed genes

Map locations of 165 of the 170 genes differentially expressed in IBD are shown in Table 1 indicating clustering of several of them within IBD susceptibility regions. Within *IBD2* (12p13.2–q24.1) we found *ATPase2B1* and *CRADD* (CASP2 and RIPK1 domain containing adapter with death domain) downregulated in CD and UC, respectively. Although *IBD1*

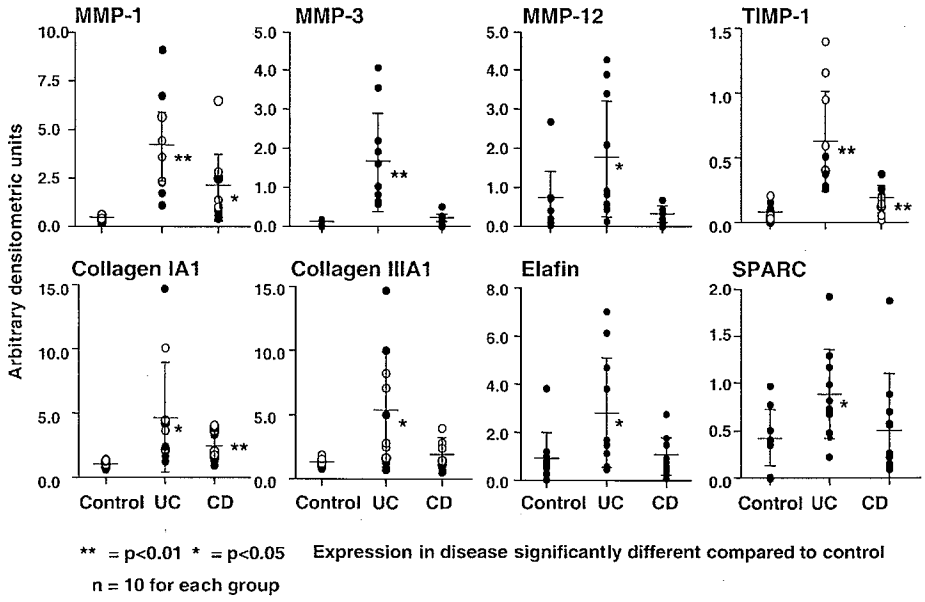

Figure 2. RT–PCR of selected transcripts on individual patients. Patient to patient variability in transcript abundance was assessed by RT–PCR of *MMP-1*, *MMP-3*, *MMP-12*, *TIMP1*, *COL1A1*, *COL3A1*, *elafin* and *SPARC* on individuals ($n = 10$) from the control, UC and CD categories. RT–PCR analysis for *MMP1*, *COL1A1 and COL3A1* transcripts was done prior to gene expression profiling. β-actin was used as an internal control and the number of PCR cycles was adjusted for signals to be within the linear range of ethidium bromide-stained bands on agarose gels. Each dot represents an individual RNA sample. Open circles indicate samples that were tested before microarray gene expression profiling. The vertical bar indicates standard error of the mean and the horizontal bar the mean. *y*-axis indicates arbitrary densitometric scanning units.

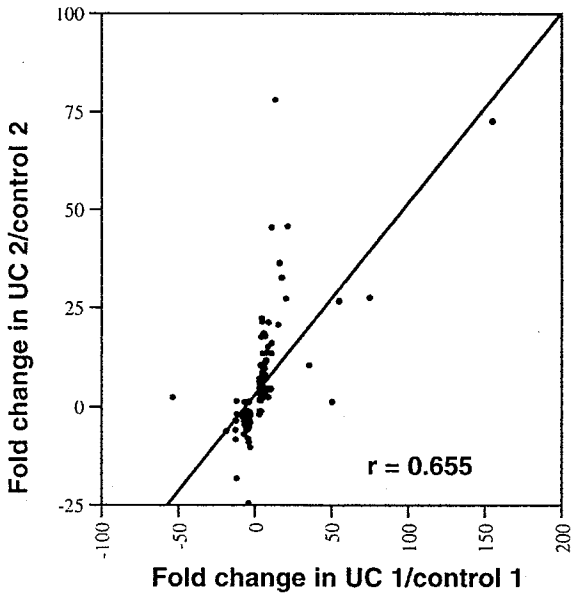

Figure 3. Experimental variability between UC 1 and UC 2. UC 1 comprises a pool of six UC target RNAs that were used throughout the study. UC 1 was compared with a control 1 profile for baseline expression and 140 genes were found to be regulated differentially at a ≥3-fold threshold. Differential expression of these 140 genes over control 2 was examined in UC 2. Fold change in gene expression in UC 2/control 2 was plotted against UC 1/control 1.

(16p12–q13) has been primarily associated with CD, among the genes probed by the arrays we did not find any that showed altered expression in CD. Because the arrays represent only 10–12% of all genes, it is possible that such putative CD markers are not included in the arrays yet. The CD profile also failed to detect any differentially expressed genes in chromosome 14q11–12 with a reportedly confirmed linkage to this condition (9,15). Another IBD susceptibility region, 19p13, contains several differentially expressed genes in UC and CD (*DF* or complement factor DM84526, *MAT8* X93036, *IF130* J03909, and *PALM* or paralemmin D87460) that are potential candidate genes. Interestingly, *MDR1* (ATP binding cassette B1, M14758) was downregulated in both UC and CD profiles. *MDR* was recently reported to be associated with UC and CD (16). *MDR1* on 7q21.1 is a candidate IBD gene (17) and *mdr1a* knockout mice develop spontaneous intestinal inflammation (18). Our observation supports the potential status of *MDR1* as an IBD candidate gene and its under-expression as indicated by our results may be due to promoter mutations. Candidate genes clustered on 6p included the over-expressed *HLA* genes in UC and others such as *UCA1B* (guanylate cyclase activator, M97496) downregulated in both UC and CD.

Gene expression profiles of UC and CD

The numeric distribution of all differentially expressed genes in UC and CD highlight shared and unique features in both diseases (Fig. 4A). An equal number of genes were over-expressed or repressed in each IBD subtype, implying that broad up- or downregulation of genes *per se* does not define

A

UC \ CD	Down-regulated	No significant change	Up-regulated
Down-regulated	15	50	0
No significant change	14	6894	15
Up-regulated	0	58	18

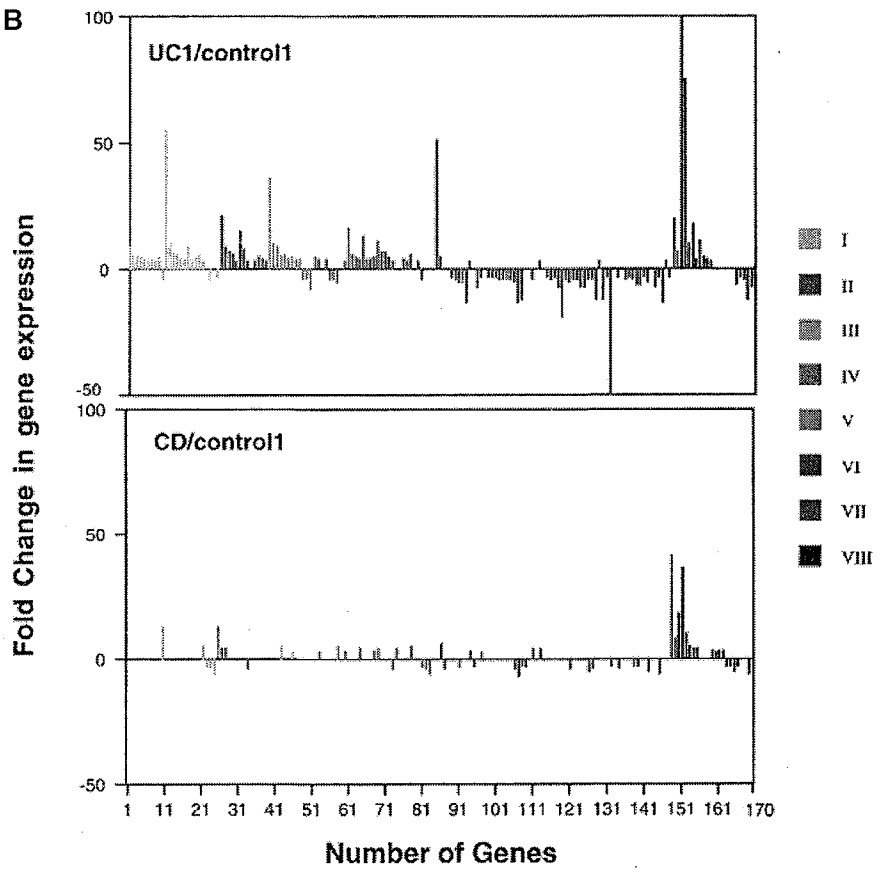

Figure 4. (**A**) Numeric distribution of differentially regulated genes in UC (red), CD (blue) and both (black). 'Downregulated' designates genes that are downregulated compared with the control 1 profile at ≥3-fold level; 'upregulated' indicates genes that are over-expressed compared with the control 1 profile at ≥3-fold level. 'No significant change' indicates genes that are expressed within the 3-fold level. Overall, 50 genes are downregulated and 58 upregulated uniquely in UC 1, whereas 14 are downregulated and 15 upregulated uniquely in CD. (**B**) Expression profiles of genes at >3-fold level in UC and CD compared with control. The vertical bars indicate increase or decrease in gene expressions in UC 1and CD compared with gene expression in control 1 at ≥3-fold level. Genes were grouped into eight clusters based on their structural and functional relatedness. Clusters in this figure correspond to those in Table 1.

these diseases. Of note, not a single gene was upregulated in one and downregulated in the other form of IBD. Furthermore, 20% of differentially regulated genes were common to both forms of IBD, probably reflecting common events secondary to inflammation. Nevertheless, 108 and 29 differentially expressed genes unique to each IBD subtype spell distinctive disease signatures for UC and CD that underscore fundamental differences in their pathogenesis.

We assigned the differentially expressed genes to eight functional clusters (Fig. 4B and Table 1) and identified key differences in gene expression in UC and CD. We noted significant over-expression of *HLA II* and immunoglobulin genes in

Table 1. Differentially expressed genes

Gene name	Function	Increase		Decrease		Chromosome	Cytogenetic	Interval
		UC	CD	UC	CD			
I. HLA and immune function genes								
HLA-DPB1 (M57466)	Antigen presentation	**9**				6	6p21.3	D6S1558–D6S1616
MHC IIβ W52 (HG3576-HT3779)	Antigen presentation	**6**				6	6	
MHC Dγ (M28590)	Antigen presentation	**5**				6	6	
HLA-DRB1 (M33600)	Antigen presentation	**5**				6	6p21.3	D6S1558–D6S1616
HLA-DR a (X00274)	Antigen presentation	**4**				6	6	D6S1558–D6S1616
HLA-DMA (X62744)	Antigen presentation	**4**				6	6p21.3	D6S1558–D6S1616
HLA-DR2-Dw12 DQw1b (M16276)	Antigen presentation	**4**				6	6	D6S1558–D6S1616
HLA-D II DQw1.1 b (X03068)	Antigen presentation	**3**				6	6	D6S1558–D6S1616
CD74 (M13560)	Antigen presentation	**5**				5, 14	5q32	D5S470–D5S487; D14S65–qTEL
CD9 (M38690)	Immune-response			4		12	12p13	D12S99–D12S358
IGHG3 (M87789)	Antibody production	**55**	**13**			14	14q32.33	D14S65–qTEL
Ig λ gene cluster (X57809)	Antibody production	**11**				22	22q11.1–q11.2	D22S420–D22S280
Ig γ chain (M63438)	Antibody production	**7**				2	2	D2S388–D2S113
Ig κ light chain (X72475)	Antibody production	**6**				12	12	D12S78–D12S79
IgA2 (S71043)	Antibody production	**4**				14	14	D14S65–qTEL
Ig λ-like polypeptide 3 (M34516)	B-cell development	**4**				22	22	D22S1144–D22S280
Ig λ-like polypeptide 2 (L02326)	B-cell development	**9**				22	22q11.2	
Ig receptor (X73079)	Immunoglobulin secretion	**4**				1	1q31–q41	D1S306–D1S491
T-cell receptor b cluster (X00437)	Immune-response	**4**				7	7q35	D7S655–D7S686; D7S2450–D7S550
IFN-γ inducible protein 30 (J03909)	Immunomodulatory	**6**				19	19p13.1	D19S899–D19S407
IFN-γ inducible protein 16 (M63838)	Immunomodulatory	**3**				1	1q12-qter	D1S514–D1S2844
IFN-γ inducible MTAP44 (D28915)	Microtubular aggregate protein		**5**			1	1	D1S203–D1S2865
Interferon-stimulated protein 15 (M13755)	Unknown			4	3	1	1	pTEL–D1S468
Interleukin 2 receptor g chain (D11086)	Immunomodulatory				3	X	Xq13.1	DXS983–DXS995
Complement D component (M84526)	Antigen presentation			3	6	19	19	pTEL–D19S413
II. Chemokines, cytokines and growth factors								
Interleukin-8 (MDNCF) (Y00787)	Chemotaxis	**21**	**13**			4	4q13–q21; 14	D4S392–D4S2947; D14S1066–D14S265
Interleukin-8 (M28130)	Chemotaxis	**9**	**4**			4	4q13–q21; 14	D4S392–D4S2947; D14S1066–D14S265
Interferon-inducible (X57351)	Chemotaxis	**7**	**4**			11	11	pTEL–D11S1318
Small inducible cytokine A4 (J04130)	Lymphocyte activation/ inflammatory	**6**				17	17q21	D17S933–D17S800
Small inducible cytokine A2 (M69203)	Lymphocyte activation/ inflammatory	**3**				17	17q21	D17S933–D17S800
GRO1 (X54489)	Growth-regulatory/inflammatory	**15**				4	4q21	D4S392–D4S2947
GRO2 (M57731)	Growth-regulatory/inflammatory	**8**				4	4q21	D4S400–D4S1534
GRO3 (X53800)	Growth-regulatory/inflammatory	**3**				4	4q21	D4S400–D4S1534; D4S392–D4S2947
TNF receptor member 1A (M58286)	Inflammatory				4	12	12p13.2	D12S99–D12S358
Interleukin-6 (X04602)	Inflammatory	**3**				7	7q21	D7S493–D7S673
Interleukin-1β (X04500)	Pro-inflammatory	**5**				2	2q14	D2S293–D2S121
Interleukin-1 receptor antagonist (X53296)	Anti-inflammatory	**4**				2	2q14	D2S293–D2S121
Growth hormone 2 (J03756)	Placental lactogen	**3**				17	17q22–q24	D17S794–D17S795
III. Inflammatory mediators								
Neutrophil lipocalin (S75256)	Bind lipophilic ligands	**36**				?		
Lipocalin 2 (X99133)	Bind bacterial lipophilic ligands	**10**				9	9q34	D9S1821–D9S159
Nitric oxide synthase 2 (X85771)	Pro-inflammatory	**9**				10	10	D10S1786–D10S541
Super oxide dismutase 2 (X65965)	Pro-inflammatory	**5**				6	6q25.3	
Phospholipase A2gp IIA (M22430)	Pro-inflammatory	**6**	**5**			1	1p35	

continued overleaf

Table 1. *Continued.*

Gene name	Function	Increase		Decrease		Chromosome	Cytogenetic	Interval
		UC	CD	UC	CD			
Annexin A5 (U01691)	Phospholipase 2 inhibitory	**4**				4	4q28–q32	D4S2945–D4S430
Serum amyloid A1 (X51441)	Acute phase response	**5**				11	11p15.1	D11S1307–D11S1359
Serglycin (X17042)	Inflammatory	**4**	**3**			10	10q22.1	d10S210–D10S537
Lysozyme (M21119)	Bacteriolytic enzyme	**4**				12	12	D12S83–D12S350
KIAA0106 (D14662)	Unknown			4		1	1	D1S210–D1S2640
Metallothionein 1H (X64177)	Sequestering/detoxifying metal ions			4		16	16q13	
Metallothionein 1G (J03910)	Sequestering/detoxifying metal ions			8		16	16q13	D16S408–D16S514
IV. Cancer-related genes								
Epithelial protein DD96 (U21049)	Upregulated in carcinoma	**5**				1	1	D1S2843–D1S417
S100 Ca-binding protein A11 Calgizzarin (D38583)	Upregulated in colorectal cancer	**4**				7, 4, 1	1q21	D7S529–D7S484; D4S1615–D4S1579
DRAL (L42176)	LIM-domain protein		**3**			2	2q12–q14	D2S113–D2S176
Growth arrest specific 3 (D11428)	Growth regulation	**4**				17	17p12–p11.2	D17S804–D17S799
MAX-interacting protein 1 (L07648)	MYC putative tumor suppressor			4		10	10q24–q25	D10S597–D10S1681
Downregulated in adenoma (L02785)	Intestinal chloride transport			4		7	7q31	D7S2420–D7S523
Selenium binding protein 1 (U29091)	Anticarcinogenic effects of selenium			5		1	1q21–q22	D1S514–D1S2844
V. ECM and remodeling genes								
Mucin (M22406)	Intestinal mucosal barrier		**5**			?		
Trefoil factor 1 (X52003)	Intestinal mucosal barrier	**3**				21	21q22.3	D21S1259–qTEL
MMP-12 (L23808)	Metalloelastase	**16**	**3**			11	11q22.2–q22.3	D11S1339–D11S1343
MMP-9 (J05070)	Gelatinase B	**6**				20	20q11.2–q13.1	D20S119–D20S197
MMP-1 (X54925)	Interstitial collagenase	**5**				11	11q22.3	D11S1339–D11S1343
MMP-3 (X05232)	Stromelysin -1	**4**				11	11q22.3	D11S1339–D11S1343
Elafin (L10343)	Elastase inhibitor	**13**	**4**			20	20q12–q13	D20S119–D20S197
Fibronectin 1 (X02761)	Basement membrane	**4**				2	2q34	D2S355–D2S163
Collagen 4A2 (X05610)	Basement membrane	**4**				13	13q34	D13S285–qTEL
Collagen 3A1 (X06700)	Interstitial ECM fibrillar collagen	**5**				2	2q31	D2S2257–D2S115; pTEL–D7S481
Collagen 1A2 (Z74616)	Interstitial ECM fibrillar collagen	**11**	**3**			2	7q22.1	D7S524–D7S527
Collagen 1A1 (M55998)	Interstitial ECM fibrillar collagen	**7**	**4**			17	17q21.3–q22	
Collagen 6A3 (X52022)	Interstitial ECM	**7**				2	2q37	D2S2158–D2S125
Collagen 6A2 (X15882)	Interstitial ECM	**5**				21	21q22.3	
von Willebrand factor (M10321)	Platelet adhesion	**3**				12	12p13.3	D12S99–D12S358
Adipose specific 2 (D45370)	Collagen-like				3	10	10	D10S1786–D10S541
Amelogenin (M86933)	Enamel matrix protein		**4**			Y	Yp11.2	
Keratocpithelin TGFbI (M77349)	Epithelial cell adhesive	**4**				5	5q31	D5471–D5S500
OSF-2 (Fasciclin I-like) (D13666)	Homophillic cell adhesive	**3**				13	13	D13S267–D13S1253
SPARC (J03040)	Regulation of cell-matrix interaction	**6**				5; 17	5q31.3–q32	D5S436–D5S487; D17S784–qTEL
Adducin 2 (X58199)	Spectrin-actin cytoskeletal organization		**5**			2	2p13–p14	D2S292–D2S145
Trichohyalin (L09190)	Epithelial cytoskeletal component	**3**				1	1q21–q23	D1S514–D1S2635; D1S439–D1S459
Zygin 2 (U60061)	Axonal bundling/adhesion			4		2	2	D2S367–D2S2230; D2S177–D2S119
Actin-related complex (AF006087)	Cytoskeletal				3	3	3	D3S3591–D3S1283
Cytokeratin 20 (X73501)	Cytoskeletal				4	17	17	D17S800–D17S930
Paralemmin (D87460)	Cell shape regulatory				6	19	19p13.3	pTEL–D19S413
VI. Metabolic pathways and ion transport mediators								
S-formylglutathione hydrolase (D28416)	Detoxification	**51**				13	13q14.1–q14.2	
Aldolase B (M15656)	Fructose metabolism	**5**				9	9q21.3–q22.2	D9S176–D9S279

continued opposite

Table 1. *Continued.*

Gene name	Function	Increase		Decrease		Chromosome	Cytogenetic	Interval
		UC	CD	UC	CD			
Glucagon (J04040)	Glycogenolysis/gluconeogenesis		**6**			2	2q36–q37	D2S156–D2S376
Monocarboxylic acid transporter 1 (L31801)	Lactate/pyruvate transport				4	1	1p13.2–p12	D1S418–D1S514
Oxoglutarate dehydrogenase (D10523)	Citric acid cycle			3		7	7p14–p13	D7S521–D7S2467
Alcohol dehydrogenase 1 (M12963)	Pyruvate turnover			4		4	4q21–q23	
Carbonic anhydrase 2 (Y00339)	Urea cycle (detoxification)			5		8	8q22	D8S275–D8S273
Carbonic anhydrase 4 (L10955)	Urea cycle (detoxification)			5	3	17	17q23	
Phosphoenolpyruvate carboxykinase 1 (L05144)	Gluconeogenesis			13		20	20q13.31	D20S183–D20S173
Syntaxin 4A (U07158)	Vesicular transport	**3**				?		
Chaperonin containing T-complex 1 (L27706)	Chaperone		**3**			7	7	D7S530–D7S509; D7S499–D7S2429
UDP glycosyltransferase 1 (J04093)	Glycosylation			7	3	2	2	D2S2158–D2S125
CRADD (U84388)	Caspase and RIP adaptor in apoptosis			3		12	12q21.33–q23.1	D12S327–D12S1657
Cystatin A (D88422)	Cysteine proteinase inhibitor		**3**			3	3q21	
Meprin 1A (M82962)	Metalloendopeptidase			3		6	6p12–p11	D6S1616–D6S427
Sulfotransferase 1A3 (U20499)	Sulfation of amines			3		16	16p11.2	
β glucoronosohydrolase (M15182)	Protein modification and detoxification			3		7	7q21.11	
N-acetyltransferase 1 (X17059)	Protein modification and detoxification			4		8	8p23.1–p21.3	D8S549–D8S258
Protein phophatase 2CA (M60483)	Protein phosphorylation			4		5	5q23–q31	D5S471–D5S393
UDP-glycosyltransferase 2B15 (U08854)	Protein modification and detoxification			4		4	4q13	D4S1619–D4S2947
Tetraspanin-3 (M69023)	Cell surface molecular facilitators			4		17	17q21	D17S933–D17S800
Thiosulfate sulfurtransferase (D87292)	Cyanide detoxification			5		22	22	D22S277–D22S272
Aminopeptidase N (M22324)	Intestinal peptide digestion			13	4	15	15q25–q26	D15S202–D15S157
Protective protein for b galactosidase (M22960)	Restores b-galactosidase activity			12	7	20	20q13.1	D20S119–D20S197
PAF acetylhydrolase (D63391)	Inactivates platelet activating factor				3	19	19q13.1	D19S425–D19S418
Tetranectin A (X64559)	Fibrinolysis				3	3	3p22–p21.3	D3S1260–D3S1588
Kallikrein 1 (M25629)	Kininogenesis			4		19	19q13.3	
Pyruvoyltetrahydropterin synthase (D17400)	Vitamin and cofactor biosynthesis		**4**			10	10q22.3–q23.3	D11S1347–D11S939
Ileal fatty acid binding protein 6 (X90908)	Cytosolic receptor for bile acids	**3**				5	5q23–q35	
Adipocyte fatty acid binding protein 4 (J02874)	Cytosolic receptor for fatty acids		**4**			8	8q21	
Liver fatty acid binding protein 1 (M10050)	Cytosolic receptor for fatty acids			3		11	11p15.5	
Enoyl CoA hydratase 1 (U16660)	Peroxisomal fatty acid b oxidation			4		19	19q13.1	
δ3δ2-CoA-isomerase (L24774)	Fatty acid metabolism			3		16	16p13.3	D16S521–D16S418
Acyl CoA dehydrogenase (Z80345)	Fatty acid metabolism			7		12	12q22-qter	D12S366–D12S340
Mitochondrial HMG Co A Synthase 2 (X83618)	Ketogenesis			19		1	1p13–p12	D1S4718–D1S514
Oxoacyl Co-A thiolase 2 (D16294)	Fatty acid b oxidation			4		18	18	D18S460–D18S474
Acetoacetyl-CoA thiolase (D10511)	Fatty acid b oxidation			5		11	11q22.3–q23.1	D11S1343–D11S1347
SREBP cleavage activating protein (D83782)	Cholesterol metabolism			4	4	3	3	D3S3582–D3S1588
Hydroxysteroid dehydrogenase isomerase (M77144)	Steroid metabolism			4		1	1p13.1	D1S418–D1S514
Hydroxysteroid (17-b) dehydrogenase 2 (L11708)	Steroid metabolism			7		16	16q24.1–q24.2	D16S515–D16S422
Hydroxysteroid dehydrogenase B2 (U26726)	Steroid metabolism			7		16	16q22	D16S3031–D16S3139
MAT8 (X93036)	Chloride channel regulator			4		19	19	D19S425–D19S418
Guanylate cyclase activator 2B (Z70295)	Electrolyte transport			4	5	1	1p34–p33	D1S2843–D1S417
Guanylate cyclase activator 1B (M97496)	Electrolyte transport			12	4	1/6	6p21.1	D1S2843–D1S417
Cytochrome P-450HFLa (D00408)	Electron transport (fetal form)	**3**				7	7	D7S479–D7S2545
Cytochrome C1 (J04444)	Electron transport			12		8, 7	8q24.3	D8S272–qTEL; D7S2493–D7S529

continued overleaf

Table 1. *Continued.*

Gene name	Function	Increase		Decrease		Chromosome	Cytogenetic	Interval
		UC	CD	UC	CD			
Cytochrome oxidase 5B (M19961)	Oxidative phosphorylation			3		2	2cen-q13	D2S113–D2S176
COX17 (L77701)	Cytochrome *c* oxidase assembly			54		13	13	D13S1253–D13S168
Estrogen receptor α (L38487)	Orphan receptor				3	11	11q12	D11S3913–D11S916
Mineral corticoid receptor 3C2 (M16801)	Aldosterone-responsiveness/Na transport			3		4	4q31.1	D4S1586–D4S1548
ATPase 2B1 (S49852)	Calcium-transporting ATPase				4	12	12q21–q23	D12S102–D12S327
ATPase 6S1 (D16469)	Lysosomal vacuolar proton pump			4		X, 2	Xq28	DXS1193–qTEL, D2S110–D2S312
SLC20A1 (L20859)	Phosphate transporter			3		2	2q11–q14	D2S293–D2S121
SLC26A2 (U14528)	Sulfate transporter			4		5	5q31–q34	D5S436–D5S470
Sorcin (M32886)	Multidrug resistance			6	3	7	7q21.1	D7S524–D7S657
ATP binding cassette B1 (M14758)	Multidrug resistance			6	3	7	7q21.1	D7S524–D7S657
Secreted/transmembrane protein 1 (U77643)	Unknown			3		17	17q25	
Butyrophilin 2A1 (U90543)	Release of milkfat globules			5		6	6p21.3	D6S1660–D6S1558
Retrovirus envelope (M11119)	Unknown				5	?		
Glycophorin E (M29610)	Specifies blood group M			7		4	4q28–q31	D4S1579–D4S1604; D4S1604–D4S1586
RNASE1 (D26129)	RNA degradation			3		14	14	pTEL–D14S283
Creatine kinase brain (M16364)	Energy metabolism			13	6	14	14q32	D14S65–qTEL
KIAA0367 (AB002365)	Unknown		**3**			9	9	D9S153–D9S6264
KIAA0110 (D14811)	Unknown			3		6	6	D6S1558–D6S427
VII. Antimicrobial								
Defensin 5 (M97925)	Antimicrobial peptides	**20**	**41**			8	8pter–p21	D8S277–D8S550
Defensin 6 (U33317)	Antimicrobial peptides	**7**	**8**			8	8pter–p21	D8S552–D8S549
VIII. Cell cycle regulators and transcription factors								
Regenerating islet-derived 1B (L08010)	Proliferation	**155**	**18**			2	2p12	D2S139–D2S169
Regenerating islet-derived 1A (J05412)	Proliferation	**75**	**36**			2	2p12	D2S139–D2S289
Pancreatitis-associated proteins (L15533)	Proliferation	**10**	**10**			2	2p12	D2S169–D2S139
S100 calcium binding A9 (M26311)	Inflammatory	**18**	**5**			1	1q12–q22	D1S514–D1S2635
S100P calcium binding protein (X65614)	Cell proliferation/differentiation	**4**	**4**			4	4p16	
Nicotinamide N-methyl transferase (U08021)	Xenobiotic metabolism	**11**	**4**			11	11q23.1	D11S1347–D11S939
G0/G1Switch protein 2 (M72885)	Lymphocyte differentiation	**5**				1	1	D1S491–D1S474
Hypoxia inducible factor 1A (U22431)	Hypoxia response modulation	**4**				14	14q21–q24	D14S1038–D14S290
CCAAT/enhancer binding protein (X52560)	Transcription factor	**3**				20	20q13.1	D20S109–D20S196
Nucleolar phosphoprotein P130 (D21262)	Nucleologenesis		**3**			10, 9	10	D10S603–D10S540; D9S153–D9S264
Sentrin 2 (Ubiquitin-like) (X99585)	Nuclear transport		**3**			8	8	
NF-kappa-B p65 (L19067)	REL family transcription factor		**3**			11	11q13	D11S1357–D11S913
SMARCD1 (U66617)	Sw1/SNF related chromatin regulator		**3**			12	12q13–q14	D12S333–D12S325
Cut (Drosophila) like-1 (NM_001913)	Repressor (developmental genes)				3	7	7q22	D7S479–D7S2545
G protein of adenyly cyclase S1 (M21142)	Cellular communication				3	20	20q13.2–q13.3	D20S183–D20S173
Upstream stimulatory factor 2 (AD000684)	c-fos interacting transcription factor			6	5	19	19q13	D19S425–D19S418
Basic transcription factor 2 (D14520)	GC-box binding			3	3	?		
Zinc finger protein 91 (L11672)	High expression in T-cells			4		19	19p13.1–p12	
POLR2J (L37127)	RNA polymerase II			12		7	7q22–q31.1	D7S479–D7S2420
TATA-BP associated factor (L39060)	Transcription factor complex			7	6	1	1	D1S474–D1S439
Insulin-like GF binding protein 2 (S37730)	Cell growth			3		2	2q33–q34	D2S137–D2S164

Numbers in bold type represent an increase in gene expression change.

UC (cluster I). Over-representation of chemokines, cytokines and growth factors (cluster II) and other inflammatory mediators (cluster III) in UC represented another difference between UC and CD. Over-expressed lipocalins (*HNL*, S75256, *NGAL* or *LCN2*, X99133), nitric oxide synthase (*NOS2*, X85781), superoxide dismutase (*SOD2*, X65965), phospholipase A2 (*PLA2*, M22430), serum amyloid A protein (*SAA*, X51441) and lysozyme (*LSZA*, M21119) reflect an acute destructive inflammatory component in UC. Pro-inflammatory and mitogenic *GRO* genes (X54489, M57731 and X53800 cluster II) previously implicated in melanomas were over-expressed in UC (19).

The cancer-related gene cluster (cluster IV) defined a clinically and biologically important difference between UC and CD. Consistent with the known tendency to neoplastic transformation in UC, we found several cancer-related genes differentially regulated in UC, but not CD. For example, *DD96* (U21049), expressed at low levels in normal epithelium, but over-expressed in lung, breast and colon carcinoma (20), was over-expressed in UC. *MXI1*, (L07648), a negative regulator of *myc* and a putative tumor suppressor gene (21), was down-regulated in UC, as was *DRA* (L02785), whose absence is associated with proliferation and neoplastic transformation of the crypt epithelium (22).

Compared with CD, considerably more extracellular matrix (ECM) genes (cluster V, *fibronectin 1*, X02761, *COL4A2*, X05610, *COL1A2*, Z74616 and *COL1A1*, M55998) were over-expressed in UC (cluster V). *vWF* (M10321), *COL6A3* and *COL6A2*, (X52022, X15882) were both upregulated in UC. ECM genes may be induced by the regulatory genes *TGF β(M77349) OSF-2 (D13666) SPARC (J03040)*, all of which were over-expressed in UC. Several matrix metalloproteinase (MMP) genes (*MMP-1*, *-3*, *-9* and *-12*, X54925, X05232, J05070 and L23808) were also over-expressed in UC, possibly in response to induction by *SPARC* (23). Cluster VI includes a large number of metabolic enzymes and ion transport mediators that were, for the most part, under-expressed in UC (47 compared with 16 in CD).

An important distinction between UC and CD was the strong over-expression of *DEFA5* and *DEFA6*, two inducible natural antimicrobial peptide genes in CD (cluster VII). The gene for defensin 5 was the single highest over-expressed gene in the CD profile, twice as high as the UC profile.

Finally, a number of cell-cycle regulators and transcription factors were also regulated differently in each form of IBD (cluster VIII). This cluster contained some of the most highly over-expressed genes in both forms of IBD. The *REG1B* and *REG1A* (regenerating islet-derived protein genes, L08010, J05412, cluster VIII) were strikingly over-expressed, particularly in UC, whereas *PAP* (pancreatitis-associated protein, *L15533*), another member of the *REG* family, was only moderately less upregulated in UC and CD.

DISCUSSION

Ulcerative colitis and CD are two related yet different forms of chronic intestinal inflammation resulting from the interaction of multiple gene products. Due to the complexity of both forms of IBD, investigation of relevant gene products has been focused primarily on inflammation and immune response genes (3). Such a narrow approach has intrinsic limitations and prevents reaching a solid grasp of all the genes that are potentially relevant to IBD pathogenesis. The objective of this investigation was to review the entire repertoire of available transcripts and elucidate all differentially regulated transcripts in representative colonic tissue using high-density oligonucleotide microarrays. With this innovative approach we hoped to identify expression patterns that would differentiate the two diseases, unravel novel aspects of UC and CD pathogenesis, identify genes not hitherto associated with IBD and eventually identify novel candidate susceptibility genes. We chose to use RNA from whole colonic tissue, which comprises heterogeneous cell types, with the specific purpose of gaining a global and representative insight into all cellular changes associated with IBD pathogenesis.

This highly innovative and comprehensive approach is not free of limitations. For instance, the pooling strategy could mask some degree of disease heterogeneity, but analysis of individual patient samples would have made this study economically prohibitive. In addition, several of the detected gene expression variations may reflect secondary rather than primary abnormalities, which, nevertheless, are still highly relevant to pathogenesis in life-long conditions such as IBD, where late events may be totally unrelated to early etiopathogenic events (3). Lending legitimacy to this approach and supporting the validity of our results is a recent study by Dieckgraefe *et al.* (24). Utilizing the same oligonucleotide arrays on pooled UC tissue these authors also found similar patterns of over-expression. Thus, 28% (21/74) of the over-expressed genes were also detected in our analysis. Most noticeably, the same genes, namely the *REG* family, *MMP*s, *defensin 5* and *S100* were the most over-expressed genes in both studies.

After ensuring the reproducibility and representativeness of the microarray methodology, the expression profiling of UC and CD revealed that of all the differentially expressed genes, only 19% (33/170) were shared by both diseases. These similarly regulated transcripts may be indicative of shared pathogenic events secondary to chronic inflammation. More notable was the observation that a large number of changes in gene expression were unique to each type of IBD. Thus, expression of 64% (108/170) and 17% (29/170) of genes was uniquely altered in UC and CD, respectively, demonstrating clear distinctions between these two diseases.

In addition to the known IBD susceptibility chromosomes, the majority of the differentially regulated genes tended to cluster on certain chromosomes, such as 4 and 17, with few differentially expressed genes on 15 and 18. Differentially expressed genes on chromosomes 4 and 17 may be indicative of other as yet unidentified IBD loci. Recently, expression profiling has been successfully used to complement conventional genetic approaches to identifying monogenic disease genes, such as *Cd36* (FAT) responsible for insulin-resistance syndrome in the rat hypertension model (25). A similar approach could be most effective in identifying function-based associations in complex multigenic diseases such as IBD, since current genetic mapping identifies regions that are still large enough to span hundreds of genes. While the implicit assumption has been that in multigenic diseases there is one gene per genomic region identifiable by positional cloning, the hypothesis that multiple genes in each region contribute to disease risk is also valid. Our data show multiple differentially regu-

lated genes within one location, providing support for this second hypothesis and clues to new candidate genes.

Due to the early nature of this study and the large number of over- and under-expressed genes in each cluster, it is presently impossible to properly interpret the global biological significance of all detected variations. Nonetheless, some limited and cautious speculations can be put forward based on current understanding of UC and CD pathophysiology.

Over-representation of several HLA II transcripts in UC implies gain rather than loss of immune function and an abnormal immune regulation in disease pathogenesis. The UC profile also emphasizes strong proliferative and regenerative responses, dedifferentiation and neoplastic propensity. A widespread downregulation of genes involved in protein, lipid, carbohydrate metabolism and various ion-transport is indicative of a major disruption in cellular homeostasis and energy utilization in UC. Furthermore, downregulation of the citric acid cycle and oxidative electron transport indicate anaerobic conditions favoring glycolysis and lactate accumulation. This broad repression of metabolic pathways revives an earlier hypothesis that energy deficiency of the colonic epithelium is a significant factor in UC pathogenesis (26).

The over-expression of antimicrobial defensins was dominant in the CD profile. Since defensins mediate innate mucosal defenses, such over-expression lends particularly strong support to the current theory of a pivotal role for the enteric flora in CD pathogenesis (27). Defensins are also T cell chemotactic and may help sustain recruitment of T cells in the CD mucosa (28).

Tissue damage is fundamentally different in UC and CD (1). Despite higher expression of ECM genes in UC, connective tissue deposition and stricture formation are features of CD. Our results suggest that this may be due to distinctive regulation of ECM and remodeling genes in each form of IBD. In UC, a rapid turnover of ECM proteins by the elevated MMPs could limit excessive ECM deposition, reducing the risk of fibrotic changes. In contrast, fibrostenotic events in CD may arise from limited ECM remodeling.

In summary, the results show that the coordinated activity of multiple immune, inflammatory, microbial and metabolic genes is profoundly altered in IBD. The identification of novel differentially expressed genes, such as *REG*, *COL6A2* and *COL6A3*, *vWf*, *MMP-12*, *elafin*, *MDR1*, *DF* and *PALM*, points to as yet unexplored pathobiologies and IBD-predisposing candidate genes. Future gene expression profiling of endoscopic biopsy tissue during the clinical evolution of IBD will enable the compilation of biologically relevant co-regulated gene clusters (29), which could uncover the triggers of disease initiation and elucidate the dynamics of disease progression.

MATERIALS AND METHODS

Tissue selection

Samples were derived from surgically resected colonic tissue from six UC set 1, six UC set 2, six CD and six control patients, each group consisting of three men and three women. The ages of UC 1 and UC 2 patients ranged from 32 to 74 years (average 49 years) and 28 to 72 years (average 51 years), respectively. The CD group ranged from 25 to 86 years (average 47 years). Classical clinical, radiologic and endoscopic criteria were used to confirm all diagnoses and all patients had comparable moderately severe clinical disease (30,31). A gastrointestinal pathologist with special expertise in IBD analyzed the tissues in a blinded and random fashion, and those displaying a moderately severe degree of inflammation were selected. To ensure comparability of the RNA samples special care was taken to select tissues with similar amounts and types of inflammatory infiltrates. Control subjects ranged from 58 to 70 years (average 65 years), and were operated for colon cancer (n = 4), diverticular disease (n = 1) and cecal polyp (n = 1). Tissues used for control RNA extraction were obtained from at least 10 cm away from the area of pathology and all were histologically normal. The project was approved by the Case Western Reserve University Institutional Review Board.

RNA extraction and preparation of target biotinylated cRNA

Fresh full-thickness colonic tissues were used. Total RNA was separately extracted from each sample using the guanidinium thiocyanate method (32) and then pooled into each group (UC, CD and control). The extracted RNA was used for RT–PCR or to prepare biotinylated cRNA for hybridization to microarrays.

Poly(A)$^+$ RNA purification, biotin labeling of cRNA and hybridization were performed according to manufacturers' protocols. Double-stranded cDNA (ds-cDNA) was synthesized from 1 μg of poly(A)$^+$ RNA using the Superscript Choice system (Gibco BRL) and an HPLC-purified T7 RNA polymerase promoter containing an oligo(dT) tail (Genset). The ds-cDNA was extracted by phenol/chloroform/isoamyl alcohol and recovered by ethanol precipitation. *In vitro* transcription was performed using 1.0 μg of ds-cDNA template and a T7 Megascript Kit (Ambion) in the presence of biotin-labeled CTP, UTP (bio-11-CTP and bio-16-UTP from Enzo Diagnostics) and unlabeled ATP, CTP, GTP and UTP. A RNeasy affinity column (Qiagen) was used to purify biotin-labeled cRNA.

Hybridization of cRNA to oligonucleotide microarrays

We initially used Affymetrix HuGene FL set 900160 containing 7070 genes/ESTs distributed over subarrays A, B, C and D. This array set contained all GenBank genes and ESTs deposited in the database prior to 1997 (http://www.affymetrix.com/). The UC 2 and control target RNA were profiled using the HuGene FL array 900183, with all genes/ESTs on one array. Since two array types were used, the control RNA was hybridized to each array type to generate a control 1 profile for comparison with UC 1 and CD and a control 2 profile for comparison with UC 2.

The biotinylated cRNA was fragmented randomly to 35–200 bases by incubation at 94°C for 35 min. At a final concentration of 0.05 μg/μl, fragmented cRNA was added to hybridization buffer (100 mM MES, 1 M Na^+, 20 mM EDTA and 0.01% Tween-20) containing 50 pM control oligonucleotide B2, control cRNA cocktail, 0.1 mg/ml herring sperm DNA and 0.5 mg/ml acetylated BSA. Target samples were hybridized to the arrays at 45°C overnight in a GeneChip hybridization oven 640 (Affymetrix) set to 60 revolutions per minute, washed and stained with streptavidin–phycoerythrin and read at a resolution of 6 μm with a Hewlett-Packard GeneArray Scanner.

Microarray data analysis

The Affymetrix absolute analysis algorithm (v3.1) was used to analyze scanned images. Detailed protocols were provided by the manufacturer and have been described previously (33). The scanned images were visually inspected using criteria provided by the manufacturer to assess uniform hybridization. The 'noise' level (500–600 fluorescence intensity units) per chip was within the acceptable range of the manufacturer's protocol. We employed global scaling using all probes to set the average intensity to an arbitrary target intensity of 150 as recommended by the software. Each hybridization was first analyzed using the Absolute Analysis Software and then by Comparative Analysis to compare UC and CD with controls, respectively, as baseline. The absolute analysis yielded the average fluorescence difference and an absolute call of absent or present for each transcript. In the comparative analysis, transcripts were considered as significantly altered over control when the ratio of average fluorescence difference of experimental to control was ≥3-fold and the average fluorescence difference change was greater than 100 arbitrary units. The latter criteria minimized selection of genes that showed large fold changes over a control involving weak absolute responses (low average fluorescence differences). The complete gene expression profile data may be viewed at http://www.jhmi.edu/~schakrav. The LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink/) database and its links to Unigene, OMIM, GenBank and PubMed databases were used to identify all known human structural homologs, functions and chromosome location. Information on genes contributing to common biochemical metabolic pathways was obtained from Biochemical Pathways (34).

RT–PCR analysis

Total RNA (1 μg) was reverse-transcribed to synthesize cDNA. β-actin was used as an internal control. The amount of cDNA, as judged by the intensity of a control β-actin signal, was comparable in all samples. Multiple reactions with varying numbers of PCR cycles were run for each transcript and one within the linear range of band intensity of ethidium bromide-stained agarose gels was chosen for each transcript. Primers used to amplify specific gene products were as follows: *COL1A1* (forward, 5′-GGC GGC CAG GGC TCC GAC CC-3′, reverse, 5′-AAT TCC TGG TCT GGG GCA CC-3′), *COL3A1* (forward, 5′-CCC AGA ACA TCA CAT ATC AC-3′, reverse, 5′-CAA GAG GAA CAC ATA TGG AG-3′), *MMP-1* (forward, 5′-GGT GAT GAA GCA GCC CAG-3′, reverse, 5′-CAG TAG AAT GGG AGA GTC-3′), *MMP-3* (forward, 5′-GTT AGG AGA AAG GAC AGT GGT CCT G-3′, reverse, 5′-GGC ATA GGC ATG GGC CAA AAC ATT-3′), *MMP-12* (forward, 5′-TCA CGA GAT TGG CCA TTC CTT-3′, reverse, 5′-TCT GGC TTC AAT TTC ATA AGC-3′), *elafin* (forward, 5′-GCA GCT TCT TGA TCG TGG TG-3′, reverse, GCC GTG GGC ATC CTG AAT GGG-3′), *TIMP1* (forward, 5′-AGT CAA CCA GAC CAC CTT ATA CCA-3′, reverse, 5′-TTT CAG AGC CTT GGA GGA GCT GGT-3′) and *SPARC* (forward, 5′-TGA GAA TGA GAA GCG CCT GGA-3′, reverse, 5′-TTG GGG GAA ACA CGA AGG GGA-3′). PCR conditions comprised a hot start at 94°C for 5 min, followed by the sequence of 94°C for 30 s, annealing at 54–56°C for 60 s and extension at 72°C for 90 s. The products were run on a 1% TAE agarose gel with 0.25 μg/ml ethidium bromide and quantified by densitometric scanning on a Bio-Rad Gel Doc 1000. Statistically significant differences between control and IBD samples were determined using the Mann–Whitney non-parametric *t*-test and a P value of <0.05.

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Exhibit E

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Pouchitis After Ileal Pouch-Anal Anastomosis: A Pouchitis Disease Activity Index

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• *Objective*: To develop a "Pouchitis Disease Activity Index" (PDAI) and to compare it with other diagnostic scoring systems for pouchitis.

• *Design*: We compared patients who had an optimal outcome with those who had a poor result attributable to recurrent pouchitis after ileal pouch-anal anastomosis (IPAA) for ulcerative colitis at the Mayo Clinic.

• *Material and Methods*: We evaluated the applicability of a PDAI that quantitated clinical findings and the endoscopic and histologic features of acute inflammation in four groups of patients: (1) 10 who underwent IPAA for ulcerative colitis and had symptoms compatible with a clinical diagnosis of pouchitis, (2) 5 who underwent IPAA for ulcerative colitis and did not have pouchitis, (3) 5 who underwent IPAA for familial adenomatous polyposis and had no symptoms of pouchitis, and (4) 5 who had a Brooke ileostomy for ulcerative colitis (control group).

• *Results*: The PDAI was significantly greater in patients with the clinical features of pouchitis than it was for patients in the other three groups. All 10 patients with pouchitis fulfilled the PDAI criteria for a diagnosis of pouchitis; in contrast, only 1 of these 10 patients met the diagnostic criteria for pouchitis by application of previously established scoring systems. No asymptomatic patient qualified for a diagnosis of pouchitis by the PDAI criteria.

• *Conclusion*: The PDAI provides simple, objective, and quantitative criteria for pouch inflammation after IPAA and is more sensitive than prior scoring systems.

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FAP = familial adenomatous polyposis; IPAA = ileal pouch-anal anastomosis; PDAI = Pouchitis Disease Activity Index; UC = ulcerative colitis

Proctocolectomy is often necessary when medical therapy fails to control the symptoms of ulcerative colitis (UC). For familial adenomatous polyposis (FAP), removal of the colon is also the usual treatment. Total proctocolectomy in conjunction with an incontinent (Brooke) ileostomy was the standard approach[1] until 1969, when Kock[2] described the continent ileostomy (Kock pouch). Although this was an attractive alternative to an incontinent stoma, surgical complications—mainly those of mechanical failure of the "nipple valve"—led to abandonment of the continent ileostomy as the standard procedure.[3] Thereafter, the concept of ileoanal anastomosis plus creation of a reservoir was developed by Parks and Nicholls[4] at St. Mark's Hospital in London. Abdominal colectomy together with ileal pouch-anal anastomosis (IPAA) has subsequently become the standard surgical therapy for uncontrolled UC and FAP.[5-7] This approach has the major advantages of removing all diseased mucosa while preserving continence and transanal defecation.

A major drawback to use of IPAA is a clinical syndrome associated with mucosal inflammation of the pouch

For accompanying editorial, see page 491

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(pouchitis), a complication that occurs in 20 to 30% of patients.[7-9] Although pouchitis is common, no universally accepted criteria have been established for the diagnosis. This lack of consensus accounts for many current dilemmas, such as the variable frequency of pouchitis reported from various medical centers as well as uncertainties about the cause and the most effective treatment.[5-7]

At the Mayo Clinic, pouchitis has been defined clinically as a syndrome of frequent, watery, and often bloody stools associated with fecal urgency, incontinence, abdominal cramping, malaise, and fever; symptoms must be present for 2 or more days, and they must respond promptly to metronidazole.[5,10] At St. Mark's Hospital, pouchitis has been defined as the following triad: diarrhea, endoscopic features of inflammation in the pouch, and histologic evidence of acute inflammation.[11-13] Within this definition, a histopathologic index, which includes the features of acute and chronic inflammation, has been developed to quantify the histologic severity.[11]

For prospective studies, a quantitative "Pouchitis Disease Activity Index" (PDAI), similar to those developed for Crohn's disease[14,15] and UC,[16-18] would be useful. A pouchitis index should quantify the broad clinical features, together with endoscopic and histologic findings; the diagnosis of clinically significant pouchitis should be based on some minimal score. If widely accepted, a PDAI could facilitate comparisons among medical centers and could accurately quantify the response of pouchitis in therapeutic trials. In the current study, our goals were to develop a PDAI and to compare it with the other commonly used definitions of pouchitis.[5,10-13]

PATIENTS AND METHODS

Study Subjects.—More than 1,400 patients with UC and FAP have undergone abdominal colectomy and IPAA at the Mayo Clinic in Rochester, Minnesota. A registry, in which the progress of each patient is recorded prospectively, enabled us to contact selected patients and ask whether they would return to participate in this study.

Our goal was to compare patients who had an optimal outcome after IPAA for UC or FAP with those who had a poor outcome that was attributed to recurrent pouchitis. Patients with a Brooke ileostomy for UC served as an additional control group. An optimal postoperative outcome was defined as a stable pattern of fewer than seven nonbloody stools per day and no abdominal cramps, fecal urgency, or fever. In addition, no prior symptoms consistent with pouchitis could have been present. In contrast, pouchitis was diagnosed clinically by an increase of at least three stools per day more than the postoperative baseline; diarrhea was associated with variable symptoms of abdominal cramping, fecal urgency and bleeding, or fever.

We recruited 25 patients into the study: (1) 10 who underwent IPAA for UC and had clinical pouchitis, (2) 5 who underwent IPAA for UC and did not have pouchitis, (3) 5 who underwent IPAA for FAP and had no pouchitis, and (4) 5 who had a Brooke ileostomy for UC. To enroll these patients, we reviewed the medical records and contacted 76 patients. On the basis of the postoperative course as recorded in the registry and the proximity to our medical center, we contacted the following number of patients in the aforementioned four categories: 27, 25, 18, and 6, respectively. Subsequently, 51 patients were excluded from analysis for the following reasons: (1) they declined to participate in the study; (2) the medical history did not completely fulfill the previously described definition of an optimal postoperative outcome; or (3) at the time they were contacted, the history was not fully consistent with pouchitis.

The four study groups are characterized in Table 1. Patients with clinical pouchitis had chronic or recurrent symptoms, as itemized in our previously mentioned definition of pouchitis, that necessitated continuous or intermittent suppressive medical therapy (usually with antibiotics). The mean (±SD) number of medications used by the 10 patients

Table 1.—**Comparison of Characteristics of Patients in Four Study Groups***

Characteristic	UC and pouchitis (N = 10)	UC, no pouchitis (N = 5)	FAP, no pouchitis (N = 5)	UC, Brooke ileostomy (N = 5)	P†
Age (yr)	37 ± 8	41 ± 16	43 ± 10	62 ± 14	<0.01
Sex (M:F)	7:3	4:1	3:2	3:2	0.64
Time since closure of ileostomy (yr)	7 ± 3	4 ± 3	6 ± 2	10 ± 6‡	0.09
Extent of colitis (pancolitis:left-sided involvement)	10:0	3:2	...	5:0	0.04

*FAP = familial adenomatous polyposis; UC = ulcerative colitis.
†Determined by analysis of variance.
‡Indicates time since creation of Brooke ileostomy.

with clinical pouchitis was 3 ± 1. The medications used were metronidazole in 10 patients, ciprofloxacin hydrochloride in 6, mesalamine in 3, and other agents in 5. Suppressive therapy was discontinued at least 14 days before this study was begun. In the patients diagnosed clinically as not having pouchitis, no medications were used. The study protocol was approved by the Institutional Review Board of the Mayo Clinic, and all patients gave written, informed consent.

Assessment of Patients.—All patients were examined by one investigator (W.J.S.) for the recording of clinical symptoms, endoscopic findings, and histologic features. Patients were questioned about the extent of the previous UC, the time elapsed since takedown of the diverting ileostomy, and the past and present use of medications for pouchitis. Patients were also questioned about the usual number of stools per day after closure of the diverting ileostomy, the current number of stools per day, the presence of blood in stools, the presence of fecal urgency or abdominal cramps, and the presence of fever. These data were entered on a score sheet (Table 2).

During flexible fiberoptic examination of the ileal pouch or Brooke ileostomy, the presence or absence of each of the following endoscopic findings[11,19-21] was noted: edema, granularity, friability, loss of vascular pattern, mucous exudate, and ulceration. These data were also entered on a score sheet (Table 2). The anus was examined visually, endoscopically, and digitally to exclude the presence of outlet obstruction from anastomotic stenosis.

During endoscopic examination of the ileal pouch or Brooke ileostomy, four mucosal biopsy specimens were obtained from either the regions of active inflammation evident endoscopically or the normal-appearing mucosa if endoscopic inflammation was absent. All biopsy specimens were reviewed in a blinded fashion by one pathologist (K.P.B.) with use of the criteria described by Moskowitz and associates.[11] Scores were based on the most severe changes seen (Table 2).

In all patients, stool bacterial cultures were negative, and examination of stool specimens showed no ova, parasites, or *Clostridium difficile* toxin.

Definition of PDAI.—Before this study was initiated, we developed a scoring sheet for pouchitis, based on clinical, endoscopic, and histologic findings (Table 2). This PDAI, which incorporated our clinical experience and the observations of other investigators,[5,10-13,19-21] evaluated the global symptoms of pouchitis and the endoscopic and acute histologic features of inflammation. The clinical criteria were stool frequency, rectal bleeding, fecal urgency, and fever. The stool frequency was compared with the postoperative baseline rather than with an absolute number of stools daily. The endoscopic criteria were edema, granularity, friability, loss of vascular pattern, mucous exudate, and ulceration. For the histologic criteria of acute inflammation, mucosal infiltration by polymorphonuclear leukocytes and ulceration were graded. We arbitrarily defined clinically significant pouchitis as a total PDAI score of 7 points or more of a possible 18 points.

Table 2.—**Elements of the Pouchitis Disease Activity Index***

Criteria	Score
Clinical	
Stool frequency	
Usual postoperative stool frequency	0
1-2 stools/day > postoperative usual	1
3 or more stools/day > postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature >37.8°C)	
Absent	0
Present	1
Endoscopic inflammation	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudate	1
Ulceration	1
Acute histologic inflammation	
Polymorphonuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field (mean)	
<25%	1
25 to 50%	2
>50%	3

*Pouchitis is defined as a total score of ≥7 points.

Statistical Analysis.—Comparisons among the four patient groups were made by using analysis of variance with Neuman-Keuls post hoc *t* tests and Pearson's χ^2 test when appropriate. Differences were considered significant at the $P<0.05$ level.

RESULTS

The results of the clinical, endoscopic, and histologic evaluations are shown in Table 3. In comparison with those with no pouchitis, patients who fulfilled the clinical diagnosis of active pouchitis had a significantly greater number of stools per day, and fecal urgency was noted in 7 of the 10. Trends were also noted for stool blood and fever to be present more frequently in patients with clinical pouchitis than in those

Table 3.—**Clinical, Endoscopic, and Histologic Findings in Four Study Groups***

Finding	UC and pouchitis (N = 10)	UC, no pouchitis (N = 5)	FAP, no pouchitis (N = 5)	UC, Brooke ileostomy (N = 5)	P†
Clinical					
Normal stools (no./day)	5 ± 2	5 ± 1	4 ± 1	5 ± 1‡	0.59
Current stools (no./day)	9 ± 2	5 ± 1	4 ± 1	5 ± 1‡	<0.00
Stool blood (yes:no)	3:7	0:5	0:5	0:5	0.16
Urgency (yes:no)	7:3	0:5	0:5	0:5	<0.00
Fever (yes:no)	4:6	0:5	0:5	0:5	0.07
Endoscopic					
Edema (yes:no)	10:0	0:5	0:5	0:5	<0.00
Granularity (yes:no)	9:1	0:5	0:5	0:5	<0.00
Friability (yes:no)	6:4	1:4	0:5	0:5	0.03
Loss of vascularity (yes:no)	9:1	0:5	0:5	0:5	<0.00
Mucous exudate (yes:no)	6:4	0:5	0:5	0:5	0.01
Ulceration (yes:no)	5:5	1:4	0:5	0:5	0.08
Histologic					
Polymorphonuclear leukocyte infiltration (1-3)	1.7 ± 0.5	1.0 ± 0	0.4 ± 0.5	0.2 ± 0.4	<0.00
Ulceration (1-3)	0.4 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0.24
Lymphocyte infiltration (1-3)	2.1 ± 0.7	1.2 ± 0.4	1.4 ± 1.1	0.6 ± 0.9	0.02
Villous atrophy (1-3)	1.9 ± 0.6	1.2 ± 0.4	0.8 ± 0.4	0.2 ± 0.4	<0.00

*FAP = familial adenomatous polyposis; UC = ulcerative colitis.
†Determined by analysis of variance.
‡Indicates number of times Brooke ileostomy bag was emptied.

without pouchitis. The endoscopic findings of edema, granularity, friability, loss of vascular pattern, and mucous exudate were all significantly more common in patients with than in those without pouchitis; a trend existed for these patients to have more mucosal ulceration also. The histologic features of polymorphonuclear leukocyte infiltration, lymphocyte infiltration, and villous atrophy were all significantly more frequent in symptomatic patients, but mucosal ulceration was noted in only 4 of the 10.

The PDAI scores for all patients are shown in Table 4. The mean PDAI score was significantly higher for patients with clinical pouchitis than for the other patients; furthermore, all 10 patients met our arbitrary score for a diagnosis of pouchitis by PDAI criteria. No patient in the other groups fulfilled these criteria, and the absolute scores in these groups were low.

Other diagnostic criteria—the "pouchitis triad" and the histopathologic index[9-11] of pouchitis—are shown in Tables 5 and 6. We applied the criteria from the pouchitis triad and the histopathologic index to our current study patients (Table 7). Although all three control groups were scored appropriately as not having pouchitis, the sensitivity of these scoring systems for identifying our patients with symptoms was less than the PDAI.

Table 4.—**Results With Use of Pouchitis Disease Activity Index***

Result	UC and pouchitis (N = 10)	UC, no pouchitis (N = 5)	FAP, no pouchitis (N = 5)	UC, Brooke ileostomy (N = 5)	P†
Mean PDAI score (0-18)‡	11 ± 3	1 ± 1	0 ± 1	0 ± 0	<0.00
Pouchitis diagnosed with PDAI criteria (no. of patients)	10	0	0	0	<0.00

*FAP = familial adenomatous polyposis; PDAI = Pouchitis Disease Activity Index; UC = ulcerative colitis.
†Determined by analysis of variance.
‡See Table 2.

Table 5.—**Pouchitis Triad: Factors Used for Diagnosis***

Factor	Points
Diarrhea (≥6 stools/day)	...
Endoscopic findings of inflammation	...
Edema	
Granularity	
Contact bleeding	
Loss of vascular pattern	
Mucosal hemorrhage	
Ulceration	
Acute histologic inflammation	
Polymorphonuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field	
<25%	1
25 to 50%	2
>50%	3

*For the diagnosis of pouchitis, all three criteria must be present, as follows: ≥6 stools/day; ≥4 endoscopic findings; and ≥4 points for the acute histologic inflammation score.
Data from Moskowitz and associates.[11]

DISCUSSION

Controversies about the clinical features of pouchitis after IPAA result from imperfect definitions, which may be either too inclusive or too exclusive. A purely clinical definition has been used at the Mayo Clinic. Because many of our more than 1,400 patients live at a considerable distance from Rochester, Minnesota, regular, in-person follow-up is unrealistic.[5,10] We recognize that this approach, based on simple clinical criteria and a response to antibiotics (usually metronidazole), could include patients with recurrent Crohn's disease, pouch ischemia, anastomotic stricture, and even irritable bowel syndrome. Furthermore, patients with pouchitis who do not respond to antibiotics would be misdiagnosed. The subjective and nonspecific nature of this definition probably overestimates the frequency of occurrence of pouchitis and does not rely on systematic, clinical observations. More objective clinical, endoscopic, and histologic data are clearly needed.

In contrast, some criteria for pouchitis may be too exclusive. At St. Mark's Hospital in London, pouchitis has been defined appropriately on the basis of the triad of diarrhea, endoscopic evidence of inflammation, and histologic features of acute inflammation.[11-13] We agree that all three components should be included; however, clinical experience suggests that the severity of pouchitis encompasses a broad spectrum. Thus, the thresholds for each of the three components should not be set so high that only patients with severe pouchitis are included. For example, should a frequency of fewer than 6 stools per day fulfill the strict definition for "diarrhea" when accompanied by symptoms of local inflammation, such as rectal bleeding, abdominal cramping, and fecal urgency? Furthermore, patients with mild to moderate endoscopic and histologic inflammation (for example, with crypt abscesses) could fail to reach the diagnostic threshold for pouchitis (see Table 5). Finally, a continuous scoring system can more easily assess the quantitative response to therapy than an absolute system. Thus, the broader clinical criteria and lower thresholds for endoscopic and histologic features in the current PDAI may be more useful than the previous scoring systems.

The PDAI was based on the pouchitis triad and the histopathologic index described by other investigators.[11-13] Some important modifications were introduced, however. First, the definition of diarrhea in relationship to a postoperative baseline detects smaller increases in stool frequency. Second, we have included other logical clinical manifestations of inflammation—rectal bleeding, abdominal cramping, and fecal urgency. Moreover, as an index of generalized inflammation, fever was included. Finally, the scores for endoscopic inflammation and histologic inflammation are expressed as a continuum of 0 to 6 points rather than requiring a minimum of 4 points. These changes allow lesser degrees of endoscopic and histologic inflammation to be factored into a final score. Chronic inflammatory histopathologic features were not included in the PDAI because these changes have previously been shown to have minimal

Table 6.—**Histopathologic Index: Factors Used for Diagnosis of Pouchitis***

Factor	Points
Acute histologic inflammation	
Polymorphonuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field	
<25%	1
25 to 50%	2
>50%	3
Chronic histologic inflammation	
Chronic inflammatory cell infiltration	
Mild	1
Moderate	2
Severe	3
Villous atrophy	
Partial	1
Subtotal	2
Total	3

*For the diagnosis of pouchitis, the following must be present: an acute histologic inflammation score of ≥4 points and a chronic histologic inflammation score of ≥4 points.
From Moskowitz and associates.[11] By permission of Springer-Verlag.

Table 7.—Diagnostic Results With Use of Pouchitis Triad and Histopathologic Index in 25 Mayo Study Patients*

Diagnosis of pouchitis	UC and pouchitis (N = 10)	UC, no pouchitis (N = 5)	FAP, no pouchitis (N = 5)	UC, Brooke ileostomy (N = 5)	P†
With use of pouchitis triad‡ (no. of patients)	1	0	0	0	1.00
With use of histopathologic index§ (no. of patients)	1	0	0	0	1.00

*FAP = familial adenomatous polyposis; UC = ulcerative colitis.
†Determined by analysis of variance.
‡See Table 5.
§See Table 6.

discriminant value.[11] Theoretically, the PDAI could specify the presence of pouchitis in patients with minimal endoscopic or histologic features. Thus, the minimal PDAI criteria might be fulfilled by having a maximal clinical score of 6, normal endoscopic findings, and only mild polymorphonuclear leukocyte infiltration on histologic examination. Such a scenario, however, seems highly unlikely. In the example presented, daily rectal bleeding and urgency are unlikely to occur except in patients with inflammatory conditions such as pouchitis or Crohn's disease of the pouch. A recent report indicated that smokers are less likely to have pouchitis than are nonsmokers or former smokers,[22] similar to the situation in patients with UC.[23] Although this observation is of interest from the etiologic (and possibly the therapeutic) viewpoint, it is unlikely to be a significant confounding factor in diagnosing pouchitis or assessing the severity of pouchitis.

Of our 10 patients who had clinical, endoscopic, and histologic features of pouchitis, only 1 fulfilled the criteria for the pouchitis triad and the histopathologic index definitions of pouchitis (Table 7). The relative contributions of the three components of the pouchitis triad in our 10 symptomatic patients were as follows: 9 patients had 6 or more stools per day, 8 had an endoscopic score of 4 or more, and 1 had an acute histologic inflammation score of 4 or more. Perhaps the established threshold score for acute histologic inflammation is too high. Lack of an absolute criterion with which these definitions could be compared is a shortcoming of all disease activity indices.[14-18]

For optimal identification of differences in clinical symptoms, endoscopic inflammation, and histologic inflammation between patients with and those without pouchitis after IPAA, we selected "ideal" patient subgroups. Thus, patients with pouchitis had clinically active disease (ensured by the withdrawal of suppressive medical therapy), which was evident on the basis of both clinical symptoms and endoscopic and histologic inflammation. In contrast, patients without pouchitis had an optimal postoperative outcome, no history of symptoms consistent with pouchitis, no evidence of endoscopic and histologic inflammation, and no requirements for medications. The highly significant difference in the mean PDAI scores between patients with and those without pouchitis suggests that we successfully identified patients in these homogeneous subgroups. Because of the strict entry criteria and because of the geographic limitations of our referral practice, the number of patients enrolled in this pilot study was small, a potential limitation of the investigation. Additional studies of larger groups of patients will be necessary to validate the PDAI and to apply it to patients with a clinical course that is between an optimal outcome and chronically active pouchitis after IPAA.

CONCLUSION

The PDAI should prove useful for defining pouchitis, especially when it has been refined by prospective application to a large number of consecutive patients.

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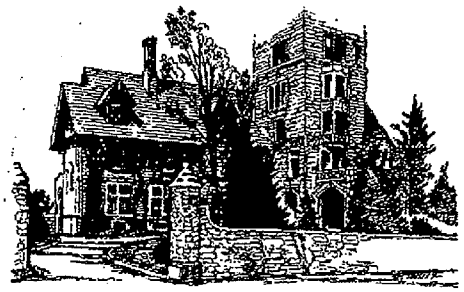

Exhibit F

7
Pouchitis: definition, risk factors, frequency, natural history, classification, and public health perspective

W. J. SANDBORN

ABSTRACT

Introduction: Abdominal colectomy with ileal pouch-anal anastomosis (IPAA) is the standard operation for most patients with ulcerative colitis (UC) who require surgery. Non-specific inflammation of the pouch (pouchitis) is the most frequent long-term complication of the IPAA surgery.

Definition: The definition of pouchitis includes clinical symptoms and endoscopic and histologic acute inflammation; but not chronic histologic inflammation. Pouchitis is perhaps best defined and measured by the Pouchitis Disease Activity Index (PDAI) criteria.

Risk factors: Risk factors for pouchitis include surgical creation of an ileal pouch, a history of UC, a history of the extra-intestinal manifestations of inflammatory bowel disease (IBD), concomitant primary sclerosing cholangitis, the presence of perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), and non-smoking. Taken together, these risk factors strongly suggest that pouchitis is a novel form of IBD specific to the ileal pouch of patients with a history of UC.

Cumulative frequency: Pouchitis occurs in about one-third of patients with an IPAA, and the cumulative risk of pouchitis is rising over time.

Natural history: Of 100 patients with an ileal pouch, 68 will not develop pouchitis, 11 will have one or two episodes, 16 will have three or more episodes, and five will develop chronic pouchitis.

Classification scheme: Patients with pouchitis should be classified according to etiology, disease activity/severity, duration, pattern, and response to treatment.

Public health perspective: Pouchitis is most likely to become an important third form of inflammatory bowel disease, given the large number of UC patients undergoing IPAA surgery and the increasing cumulative frequency of

pouchitis. Thus it seems worthwhile to improve the accuracy with which pouchitis is diagnosed and classified.

INTRODUCTION

Abdominal colectomy with ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for the majority of patients with uncontrolled ulcerative colitis (UC). The most frequent long-term complication following IPAA for UC is idiopathic inflammation of the ileal pouch, designated as pouchitis when it is clinically symptomatic. Pouchitis appears to be a unique form of recurrent inflammatory bowel disease (IBD) specific to the ileal reservoir[1]. The following review of pouchitis emphasizes the definition and diagnostic criteria, risk factors, cumulative frequency, natural history data, and a disease classification scheme for this increasingly common form of IBD.

DEFINITION AND DIAGNOSTIC CRITERIA

Clinical symptoms in patients with pouchitis invariably include increased stool frequency and may include bleeding, abdominal cramping, urgency and tenesmus, incontinence, fever, and malaise[2]. The endoscopic mucosal changes seen in pouchitis may include edema, granularity, contact bleeding, loss of the vascular pattern, mucosal hemorrhage, and ulceration within the pouch[3–4]. The mucosa of the neo-terminal ileum above the ileal reservoir should appear normal. Histologic changes of villous atrophy, crypt hyperplasia, and chronic inflammatory cell infiltration are found very frequently in biopsy specimens taken from the ileal reservoir (both from normal IPAA patients and those with pouchitis) and represent a non-specific adaptive response of the pouch mucosa to fecal stasis[4–5]. In contrast, findings of neutrophil infiltration and mucosal ulceration in ileal reservoir biopsies are relatively specific for pouchitis[4–5].

These clinical symptoms, as well as the endoscopic and histologic findings in the ileal reservoir, have been used to develop definitions and diagnostic criteria for pouchitis, including a purely clinical definition shown in Table 1 (2); a purely histologic definition shown in Table 2[4–5]; and criterion definitions which combine clinical, endoscopic, and histologic findings shown in Tables 3 and 4[4,6]. The usefulness of the purely clinical and histologic definitions of pouchitis is limited because they are not sensitive or specific[6]. The first definition used a combination of clinical, endoscopic and histologic findings, and defined pouchitis as a triad of: diarrhea; endoscopic findings of ileal pouch inflammation; and histologic findings of acute ileal mucosal inflammation, as shown in Table 3[4]. This definition is of limited usefulness because the thresholds for each

Table 1 Clinical definition of pouchitis

1. Frequent, watery, and often bloody stools.
2. Associated fecal urgency, incontinence, abdominal cramping, malaise and fever.
3. Symptoms should present for at least two days and they must respond quickly to metronidazole.

Table 2 Histopathologic index*

A. Acute histologic inflammation

1. Polymorph infiltration
   Mild = 1 point
   Moderate + crypt abscess = 2 points
   Severe + crypt abscess = 3 points
2. Ulceration per low power field
   < 25% = 1 point
   ≥ 25% ≤ 50% = 2 points
   > 50% = 3 points

A. Chronic histologic inflammation

1. Chronic inflammatory cell infiltration
   Mild = 1 point
   Moderate = 2 points
   Severe = 3 points
2. Villous atrophy
   Partial = 1 point
   Subtotal = 2 points
   Total = 3 points

A diagnosis of pouchitis requires the presence of both an acute histologic inflammation score ≥ 4 points; and a chronic histologic inflammation score ≥ 4 points

* Adapted with permission from: Shepherd NA, Jass JR, Duval I, Moskowitz RL, Nicholls RJ, Morson BC. Restorative proctocolectomy with ileal reservoir: pathological and histochemical study of mucosal biopsy specimens. J Clin Pathol. 1987;40:601–607.

of the three components are set so high that only patients with severe pouchitis are included[6]. The 'pouchitis triad' definition was later modified and renamed the Pouchitis Disease Activity Index (PDAI) as shown in Table 4[6]. This quantitative index includes multiple clinical symptoms of pouchitis (rather than just diarrhea), and expresses the endoscopic and acute histologic inflammation scores continuously rather than requiring minimum scores, thus allowing a diagnosis of pouchitis in patients with symptoms of mild or moderate severity. The PDAI has now been used in a variety of studies investigating the etiology and pathophysiology of pouchitis as well as in drug therapy studies, and appears to be a useful means by which to quantify pouchitis disease activity[7–10].

RISK FACTORS

Multiple risk factors for the development of pouchitis following colectomy have been identified and are shown in Table 5. First, distal ileal inflammation is frequently found in patients with both the Kock continent ileostomy and the IPAA ileal reservoir, and is called pouchitis[11–12]. In contrast, distal ileal inflammation is only rarely found in patients with an end ileostomy, where it is called prestomal ileitis[13]. Thus the surgical creation of an ileal reservoir is a risk factor for developing distal ileal inflammation (pouchitis). Second, the frequency of

Table 3 Pouchitis triad*

A. Diarrhea (≥ 6 stools/day)

B. Findings of endoscopic inflammation
Edema
Granularity
Contact bleeding
Loss of vascular pattern
Mucosal hemorrhage
Ulceration

C. Acute histologic inflammation

1. Polymorph infiltration
Mild = 1 point
Moderate + crypt abscess = 2 points
Severe + crypt abscess = 3 points

2. Ulceration per low power field
< 25% = 1 point
≥ 25% ≤ 50% = 2 points
> 50% = 3 points

A diagnosis of pouchitis requires the presence of all three criteria: ≥ 6 stools/day; ≥ 4 endoscopic findings; acute histologic inflammation score ≥ 4 points.

* Adapted with permission from: Moskowitz RL, Shepherd NA, Nicholls RJ. An assessment of inflammation in the reservoir after restorative proctocolectomy with ileoanal ileal reservoir. Int J Colorectal Dis 1986;1:167–174.

pouchitis following IPAA is much lower in familial polyposis patients than in UC patients[14]. Third, there is a correlation between pouchitis and a history of extra-intestinal manifestations of IBD, particularly arthritis[2,15]. Fourth, primary sclerosing cholangitis (PSC) is strongly associated with pouchitis. The cumulative risk in the Mayo Clinic Series for developing pouchitis in UC patients with PSC at one, five, and ten years postoperatively is 22%, 61%, and 79%; whereas the cumulative risk of pouchitis in UC patients without PSC is 15%, 36%, and 46% (Figure 1)[11]. Fourth, in most studies, the prevalence of anti-neutrophil cytoplasmic antibody with a perinuclear staining pattern (pANCA) is increased in patients with pouchitis as compared to patients without pouchitis, as shown in Table 6[8,16–20]. Fifth, non-smoking conveys a marked increased risk for developing pouchitis, similar to the situation in patients with UC[21]. Taken together, these risk factors strongly suggest that pouchitis is a novel form of IBD specific to the ileal pouch of patients with a history of UC.

CUMULATIVE RISK FOR FIRST EPISODE OF POUCHITIS

There are no population-based estimations of the annual incidence and prevalence of pouchitis in patients with UC who have undergone abdominal colectomy with IPAA or Kock pouch. Furthermore, there are not even data on the annual incidence and prevalence of pouchitis in the patient populations at major

Table 4 Pouchitis disease activity index (PDAI)*

	Score
Clinical Criteria	
Stool frequency	
Usual post-op stool frequency	0
1–2 stools/day > post-op usual	1
3 or more stools/day > post-op usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency/abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature > 100°C)	
Absent	0
Present	1
Endoscopic Criteria	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucus exudate	1
Ulceration	1
Acute histologic criteria	
Polymorph infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low power field (average)	
< 25%	1
≥ 25% ≤ 50%	2
> 50%	3

Pouchitis is defined as a total PDAI score ≥ 7 points

* Adapted with permission from: Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Phillips SF. Pouchitis following ileal pouch-anal anastomosis: a pouchitis disease activity index. Mayo Clin Proc 1994;69:409–415.

Table 5 Risk factors for developing pouchitis

Risk Factor	*Explanation*
A. Ileal reservoir	Pouchitis occurs in Kock and ileoanal pouches but not in ileostomies
B. Ulcerative colitis	Pouchitis occurs in patients with UC but not in familial adenomatous polyposis
C. Extra-intestinal manifestationsof IBD	Pouchitis occurs more frequently in UC patients with extra-intestinal manifestations of IBD
D. Primary sclerosing cholangitis	Pouchitis occurs more frequently in UC patients with concomitant PSC
E. Non-smoking	Pouchitis occurs more frequently in non-smokers, similar to UC
F. pANCA	Pouchitis occurs more frequently in patients who are pANCA positive

Table 6 Peri nuclear anti-neutrophil cytoplasmic antibodies (pANCA) in pouchitis

		pANCA Positive	
Author	*Reference*	*Pouchitis*	*No pouchitis*
Sandborn	8	19/19 (100%)	9/18 (50%)
Vecchi	16–17	8/9 (89%)	6/33 (18%)
Patel	18	5/5 (100%)	24/34 (71%)
Reumaux	19	4/5 (80%)	Not Stated
Aisenberg	20	11/26 (42%)	24/42 (57%)

Table 7 Cumulative risk of pouchitis over time at major centers

	Mayo	*U MN*	*Center St. Mark*	*Sweden*	*Cleveland*
Initial Report					
Year	1985	1987	1986	1989	
Reference no.	22	23	25	27	
Patient Number (*n*)	188	116	55	100	
Pouchitis (%)	**8**	**22**	**11**	**23**	
Most Recent Report					
Year	1996	1992	1994	1993	1995
Reference no.	11	24	26	12	28
Patients (*n*)	1043	196	60	96	1005
Pouchitis (%)	**32**	**34**	**50**	**47**	25

surgical referral centers. The only data which are available are 'cumulative risk for the first episode of pouchitis' at surgical referral centers. Even these cumulative risk data are problematic because they vary with duration of follow-up, the criteria used to diagnose pouchitis, and the intensity of evaluation for pouch dysfunction. The reported cumulative risk for the first episode of pouchitis at most major centers has increased over time, as shown in Table 7[11–12,22–28]. A recent study from the Mayo Clinic reported that the cumulative risk for the first episode of pouchitis at one, five, and ten years after IPAA is 15%, 36%, and 46% respectively (Figure 1)[11]. These figures are similar to those reported for Swedish UC patients where the cumulative risk for the first episode of pouchitis at five years was 47% in patients with the IPAA and 33% in patients with the Kock ileal pouch (Figure 2)[12].

NATURAL HISTORY

It is important to review the natural history of patients with pouchitis in order to gain a perspective as to whether the increasing occurrence of this new form of IBD should lead to a reconsideration of the use of abdominal colectomy with IPAA in patients with UC. At the Mayo Clinic, in UC patients with IPAA who do not have concomitant PSC, the cumulative risk of developing at least one episode of pouchitis is 32% (this figure is not adjusted for duration of follow-up

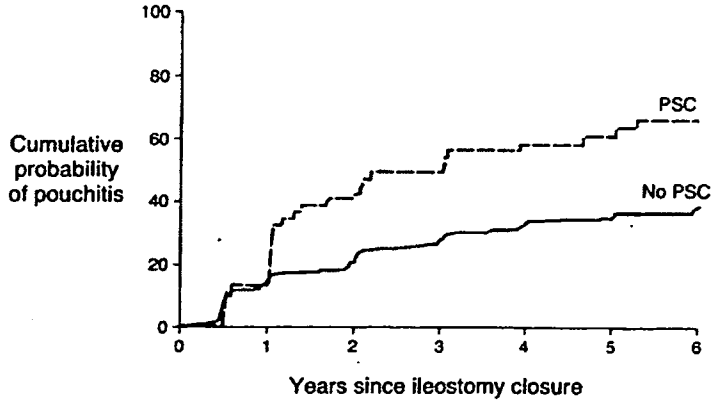

Figure 1 Occurrence of pouchitis estimated as a function of time after closure of temporary ileostomy. PSC indicates primary sclerosing cholangitis. Reproduced with permission from: Penna C, Dozois R, Tremaine W, Sandborn W, LaRusso N, Schleck C, Ilstrup D. Pouchitis after ileal pouch-anal anastomosis occurs with increased frequency in patients with associated primary sclerosing cholangitis. Gut 1996;38:234–239.

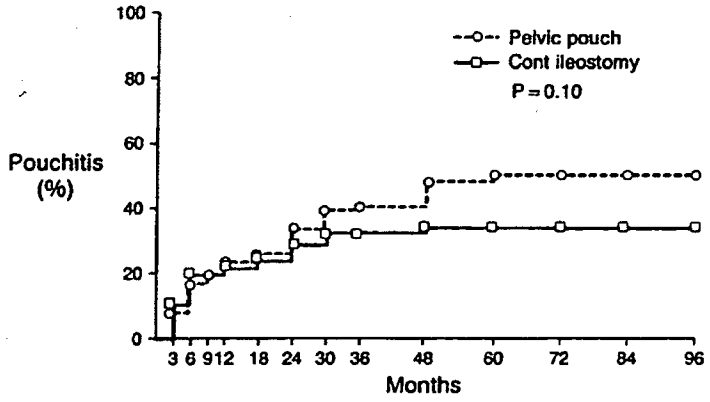

Figure 2 Cumulative risk for a first attack of pouchitis. Reproduced with permission from: Svaninger G, Nordgren S, Oresland T, Hulten L. Incidence and characteristics of pouchitis in the Kock continent ileostomy and the pelvic pouch. Scand J Gastroenterol 1993;28:695–700.

and thus represents the prevalence for a referral population)[11]. Of those patients who develop pouchitis, 36% have one or two acute pouchitis episodes which respond to treatment with antibiotics, 49% relapse more frequently (at least three acute episodes) but respond to antibiotics, and 15% require maintenance suppressive therapy and have been labeled as having 'chronic pouchitis' as shown in Figure 3[11]. Of this latter group with chronic pouchitis, almost 50% require surgical exclusion or excision of the pouch. An algorithm showing the clinical course of pouchitis in Mayo Clinic IPAA patients is shown in Figure 4. Again, similar findings are reported in Swedish UC patients with an IPAA, where 76% have a few mild episodes of pouchitis, 18% have frequently relapsing mild episodes, and 6% have severe chronic pouchitis (Figure 5)[12].

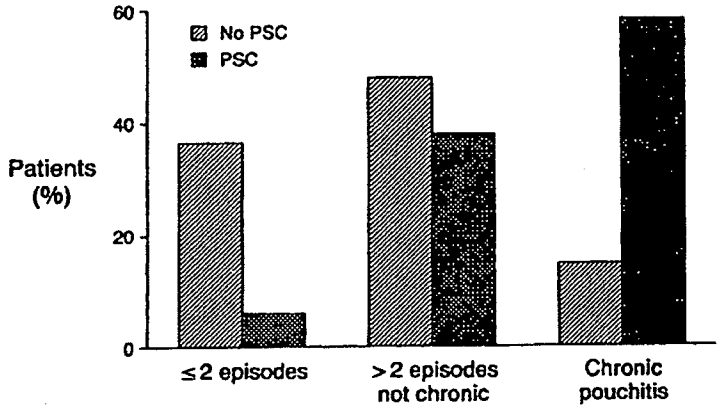

Figure 3 Pouchitis disease course after ileal pouch-anal anastomosis in patients with and without PSC. PSC indicates primary sclerosing cholangitis. Reproduced with permission from: Penna C, Dozois R, Tremaine W, Sandborn W, LaRusso N, Schleck C, Ilstrup D. Pouchitis after ileal pouch-anal anastomosis occurs with increased frequency in patients with associated primary sclerosing cholangitis. Gut 1996;38:234–239.

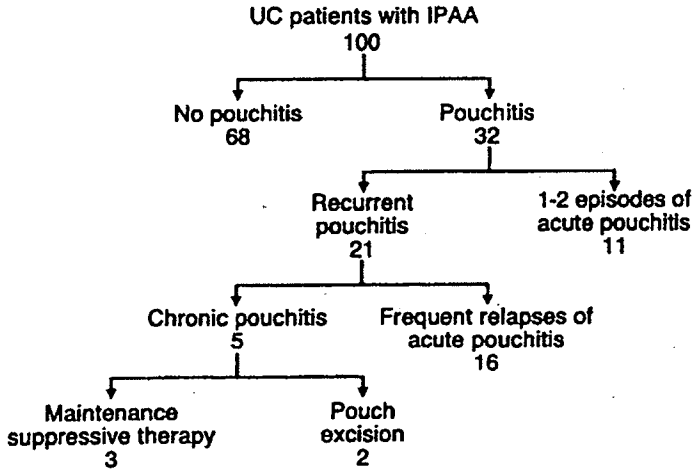

Figure 4 Clinical outcome with regard to pouchitis in 100 UC patients undergoing abdominal colectomy with ileal pouch-anal anastomosis.

DISEASE CLASSIFICATION SCHEME

The terminology utilized in the above paragraph on the natural history of pouchitis (acute versus chronic, mild versus severe, infrequent versus relapsing versus chronic) seems worth pursuing in a more formal way in an attempt to classify more accurately the clinical course of patients with pouchitis. The models for such a classification system could come from UC and Crohn's disease. There are five components which should be considered in the classification of pouchitis: etiology (idiopathic versus secondary); disease activity/severity; symptom duration; pattern; and response to treatment (Table 8). First, it is important to be certain that the patient has idiopathic pouchitis by specifically excluding secondary causes of pouchitis or pouch dysfunction such as anastomotic stricture,

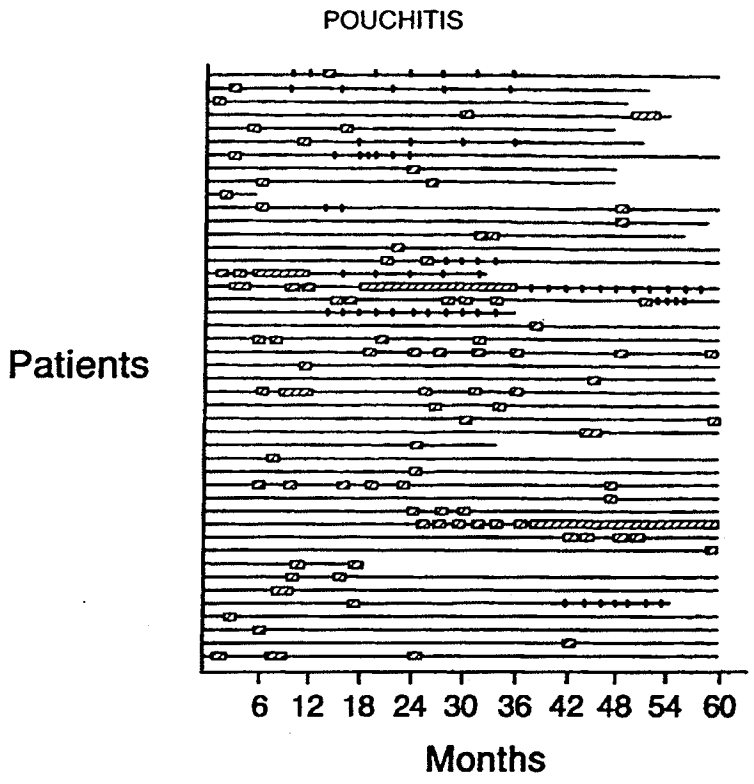

Figure 5 Pattern of pouchitis in 45 pelvic pouch patients. Clinical pattern of pouchitis during the first five years postoperatively. Short lasting attacks, responsive to treatment (—▨▨▨—); frequently relapsing, short-lasting attacks (—╫╫—); chronic continuous severe symptoms (—▨▨▨—). Reproduced with permission from: Svaninger G, Nordgren S, Oresland T, Hulten L. Incidence and characteristics of pouchitis in the Kock continent ileostomy and the pelvic pouch. Scand J Gastroenterol 1993;28:695–700.

anastomotic fistula or abscess, infectious enteritis, Crohn's disease, or 'strip pouchitis'. This latter entity occurs when the rectal remnant in an IPAA with a stapled anastomosis is the sole focus of inflammation[29]. Second, the pouchitis activity and severity should be classified as remission (no active pouchitis); mildly/moderately active pouchitis (increased stool frequency, urgency, infrequent incontinence); or severely active pouchitis (hospitalization for dehydration, frequent incontinence) (Figure 6A). Third, the duration of pouchitis needs to be quantified as acute (≤ four weeks) or chronic (> four weeks) (Figure 6B). Fourth, the pattern of the patient's pouchitis should be characterized: infrequent (one or two acute episodes); relapsing (≥ three acute episodes); or continuous (Figure 6C). Fifth, the use of and response to medical therapy should be described: none; treatment-responsive (specify medications); or treatment-refractory (specify medications).

PUBLIC HEALTH PERSPECTIVE/IMPORTANCE

The total number of patients with UC in the United States is approximately 250 000–350 000 patients, assuming a prevalence of 100–140/100 000 and a total

Table 8 Disease classification scheme for pouchitis

A. Etiology
   1. Idiopathic
   2. Secondary
      a. Anastomotic stricture
      b. Anastomotic fistula/abscess
      c. Infectious enteritis
      d. Crohn's disease
      e. 'Strip pouchitis'

B. Disease activity/severity
   1. Remission
   2. Mildly/moderately active
   3. Severely active

C. Duration
   1. Acute (≤ 4 weeks)
   2. Chronic (> 4 weeks)

D. Pattern
   1. Infrequent (1–2 acute episodes)
   2. Relapsing (≥ 3 acute episodes)
   3. Continuous

E. Response to treatment
   1. No medical therapy
   2. Treatment-responsive
   3. Treatment-refractory

United States population of 250 000 000[30–31]. The colectomy rates at ten years for patients with ulcerative colitis are in the range of 25%[32–33], and the majority of patients who undergo colectomy for UC now have an IPAA procedure[34]. Thus it is reasonable to expect that the total number of patients in the United States with an IPAA will eventually approach 60 000–90 000 patients). If 50% of these patients develop pouchitis within ten years, then the total number of patients with pouchitis will eventually approach 30 000–45 000 patients (preva-

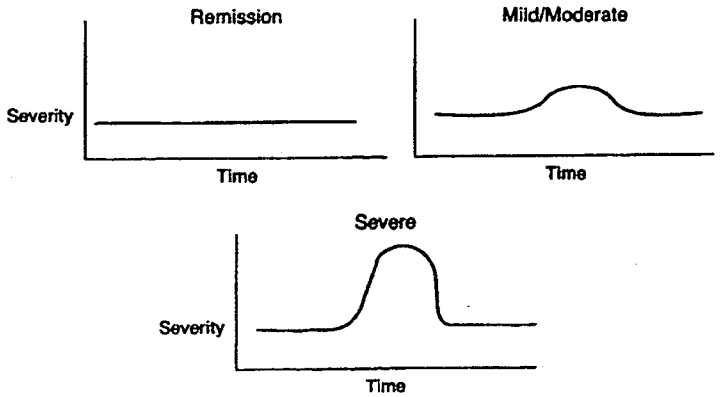

Figure 6A. Illustration of pouchitis disease activity as measured by three levels of severity (remission, mild/moderate, severe).

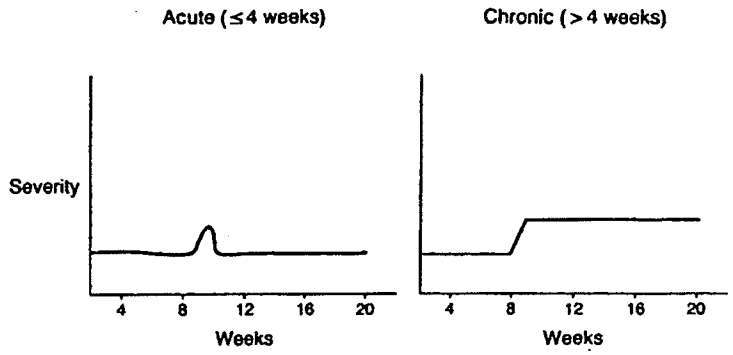

Figure 6B Illustration of pouchitis disease duration as measured by two lengths of time: acute (≤ 4 weeks); and chronic (> 4 weeks).

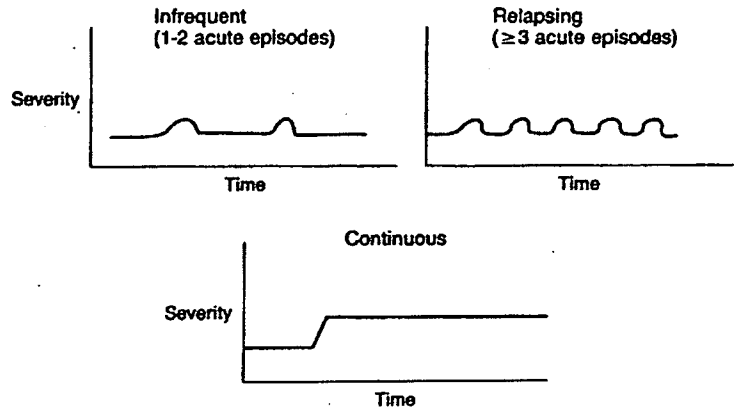

Figure 6C Illustration of three pouchitis disease patterns: infrequent (1–2 acute episodes); relapsing (≥ 3 acute episodes); and continuous.

lence of 12–18 per 100 000). Thus, pouchitis will likely become an important third form of inflammatory bowel disease. Given this strong possibility, it seems worthwhile to make every effort to improve the accuracy with which we diagnose and classify this disease.

CONCLUSION

Abdominal colectomy with IPAA is the standard operation for most patients with UC who require surgery. Pouchitis is the most frequent long-term complication of the IPAA. The definition of pouchitis includes clinical symptoms and endoscopic and histologic acute inflammation; but not chronic histologic inflammation. Risk factors for pouchitis include surgical creation of an ileal pouch, a history of UC, a history of the extra-intestinal manifestations of IBD, concomitant PSC, the presence of pANCA antibodies, and non-smoking. Pouchitis occurs in about one-third of patients with an IPAA and the cumulative risk of pouchitis is rising over time. Of 100 patients with an ileal pouch, 68 will not develop pouchitis, 11 will have one or two episodes, 16 will have three or

more episodes, and five will develop chronic pouchitis. Patients with pouchitis should be classified according to etiology, disease activity/severity, duration, pattern, and response to treatment.

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Exhibit G

Medical Management of Postoperative Complications of Inflammatory Bowel Disease: Pouchitis and Crohn's Disease Recurrence

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Surgical intervention is often required for patients with inflammatory bowel disease. Total proctocolectomy with ileal pouch–anal anastomosis is the surgical treatment of choice for patients with ulcerative colitis. The main long-term complication of this surgery is pouchitis, with 10-year cumulative incidence rates between 24% and 46%. For patients with Crohn's disease, postoperative recurrence is a significant problem, with clinical recurrence rates as high as 55% at 5 years and 76% at 15 years. Increasing evidence suggests that postoperative medical therapy has the potential to decrease the risk of postoperative Crohn's disease recurrence.

Introduction

The inflammatory bowel diseases, ulcerative colitis and Crohn's disease, are chronic medical conditions for which there is a growing list of available medical therapies. However, up to 30% of patients with ulcerative colitis and up to 70% of patients with Crohn's disease require surgery at some point in their disease course [1]. Surgery for ulcerative colitis is curative and, with the advent of the ileal pouch–anal anastomosis (IPAA) procedure, a permanent ileostomy is no longer required. On the other hand, surgery for Crohn's disease is usually only a temporizing intervention because of the high rate of recurrent disease.

This review focuses on the two most common long-term complications of surgery for inflammatory bowel disease—pouchitis in patients with ulcerative colitis and postoperative recurrence in patients with Crohn's disease. Management of these conditions is emphasized.

Pouchitis

Background

Total proctocolectomy with IPAA is the surgical treatment of choice for most patients with ulcerative colitis who have medically refractory disease or who develop dysplasia. Complications after IPAA include pouchitis, pelvic sepsis, stricture formation, and incontinence. Pouchitis is the most common long-term complication, but its incidence varies with factors such as the duration of follow-up, the diagnostic criteria used to define pouchitis, and the intensity of evaluation for pouch inflammation [2,3]. Reported 10-year cumulative incidence rates in patients with underlying ulcerative colitis vary between 24% and 46% [2–4]. In contrast, the frequency of pouchitis in patients with underlying familial adenomatous polyposis ranges from 0% to 10%, suggesting that some aspect of the underlying disease predisposes to this complication [5–7].

Pouchitis is a poorly understood inflammatory process of the ileal reservoir manifested by symptoms of increased stool frequency, fecal urgency, and abdominal cramping. Occasionally, patients also develop malaise, fever, pelvic discomfort, and extraintestinal manifestations. These symptoms suggest a diagnosis of pouchitis, but ideally, this diagnosis should be confirmed by pouch endoscopy with biopsy [1,8•]. To standardize diagnostic criteria, an 18-point Pouchitis Disease Activity Index (PDAI) has been developed [9]. The PDAI calculates an overall score from three components: clinical symptoms, endoscopic findings, and histologic changes (Table 1). Pouchitis is defined by a total PDAI score of 7 or greater.

With progressive understanding of the natural history of pouchitis, a new disease classification system that is analogous to those used for other forms of inflammatory bowel disease has been proposed [10]. This classification system defines factors such as etiology (idiopathic versus secondary), disease activity (remission versus active disease), disease severity (mild, moderate, or severe), symptom duration (acute versus chronic), and patterns (infrequent episodes versus relapsing versus continuous

Table 1. The Pouchitis Disease Activity Index

Criteria	Score
Clinical	
Stool frequency	
Usual postoperative stool frequency	0
1–2 stools/d > postoperative usual	1
3 or more stools/d > postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature > 37.8°C)	
Absent	0
Present	1
Endoscopic inflammation	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudate	1
Ulceration	1
Acute histologic inflammation	
Polymorphic nuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration/low-power field (mean)	
<25%	1
25% to 50%	2
>50%	3

course). Such classification systems can help direct treatment of pouchitis. For example, most patients with acute pouchitis respond quickly to short-term antibiotic therapy, whereas patients with chronic or relapsing pouchitis frequently require chronic medical therapy.

Treatment of pouchitis

Overview

The treatment of pouchitis has been largely empiric because of a paucity of controlled trials. Uncontrolled trials have reported benefits for many therapies, including metronidazole, ciprofloxacin, amoxicillin/clavulanic acid, erythromycin, tetracycline, corticosteroid and budesonide enemas, mesalamine enemas, oral sulfasalazine and mesalamine, oral corticosteroids, allopurinol, azathioprine, and bismuth subsalicylate [11••,12]. However, only three small placebo-controlled trials and one small controlled trial comparing two active agents have reached the publication stage.

Antibiotics

Most patients with acute pouchitis respond promptly to antibiotic therapy, but 5% to 10% develop refractory or rapidly relapsing symptoms that require protracted therapy [13]. Of patients with acute pouchitis, 39% have a single acute episode that responds to treatment with antibiotics, whereas the remaining 61% of patients go on to develop at least one recurrence [5].

Metronidazole is the only antibiotic to have been studied in a placebo-controlled randomized clinical trial. Madden *et al.* [14] performed a crossover trial in which 11 patients with chronic active pouchitis each received a 2-week course of oral metronidazole (1200 mg/d) and a 2-week course of placebo. Reduction in stool frequency was noted in 73% of those patients treated with metronidazole, compared with 9% of those treated with placebo ($P<0.05$ for median change in bowel movements/d). However, metronidazole therapy did not significantly affect endoscopic or histologic scores. Based on such results and on the low cost of metronidazole ($2/2-week course), this agent has generally been considered as first-line therapy for acute pouchitis [2,11••,12].

If patients fail to respond to or cannot tolerate metronidazole, second-line therapy has generally consisted of other broad-spectrum antibiotics such as ciprofloxacin, tetracycline, clarithromycin, amoxicillin/clavulanic acid, doxycycline, or rifaximin [11••,12]. In uncontrolled trials, ciprofloxacin has shown efficacy in treatment of acute episodes of pouchitis [15]. Ciprofloxacin has also been effective when combined with rifaximin in treatment of chronic pouchitis [16]. In our randomized clinical trial, we compared ciprofloxacin to metronidazole in 16 patients with acute pouchitis [17]. Both ciprofloxacin and metronidazole led to symptomatic improvement and significant decreases in PDAI scores. However, compared with patients in the metronidazole group, patients in the ciprofloxacin group experienced significantly greater reductions in mean total PDAI score (6.9 vs 3.8, $P=0.002$), symptom subscore (2.4 vs 1.3, $P=0.03$), and endoscopy subscore (3.6 vs 1.9, $P=0.03$). None of the patients in the ciprofloxacin group experienced adverse effects, whereas three patients in the metronidazole group (33%) developed nausea, vomiting, dysgeusia, or transient peripheral neuropathy [17]. The main disadvantage to use of ciprofloxacin is that it is much more expensive than metronidazole, with a cost of $127 for a 2-week course. Based on these factors, we then performed a cost-effectiveness analysis, which demonstrated that metronidazole and ciprofloxacin have comparable cost-effectiveness ratios [18].

In clinical practice, pouchitis is often diagnosed based on symptoms alone and empirically treated with an antibiotic such as metronidazole. Patients who do not respond to initial therapy usually then undergo diagnostic testing, including pouch endoscopy. However, this treat-then-test strategy has several pitfalls, such as unnecessarily exposing some patients who do not have pouchitis to antibiotics. A recent study from The Cleveland Clinic Foundation demonstrated that symptoms in patients with IPAA do not predict endoscopic and histologic findings and that symptom assessment alone is not reliable for accurate diagnosis

of pouchitis [8•]. Twenty-five percent of patients with moderate to severe symptoms suggestive of pouchitis did not have endoscopic and histologic evidence of inflammation of the ileal pouch [8•]. Furthermore, empiric treatment with antibiotics may delay more appropriate therapy for symptomatic patients whose symptoms are caused by conditions other than pouchitis, such as inflammation of the rectal cuff.

In summary, one small placebo-controlled trial has demonstrated the effectiveness of metronidazole for the treatment of pouchitis. Uncontrolled trials have suggested benefits for several other antibiotics. A small study from our institution suggests that ciprofloxacin may have some advantages over metronidazole, and it is equally cost effective for a 2-week course of therapy. Further randomized controlled trials are required to determine the best antibiotics for treating pouchitis and to define optimal dosing and duration of antibiotic therapy for both acute and chronic pouchitis.

Probiotics

Probiotics are viable microorganisms that, when ingested, have a beneficial effect on human health. Recent interest has been shown in the use of probiotics for management of inflammatory bowel disease [19]. Proposed mechanisms of probiotic efficacy in inflammatory bowel disease include suppression of resident pathogenic bacteria, stimulation of mucin glycoprotein production by intestinal epithelial cells, prevention of adhesion of pathogenic strains to epithelial cells, and induction of host protective immune responses [19].

A recent randomized, double-blind, placebo-controlled trial evaluated the use of a probiotic named VSL-3 for maintenance therapy of acute relapsing pouchitis after induced remission using ciprofloxacin and rifaximin [20•]. VSL-3 consists of four strains of *Lactobacillus*, three strains of *Bifidobacterium*, and one strain of *Streptococcus salivarius* at high concentrations of 300 billion viable bacteria per gram. Only three of 20 patients (15%) in the probiotic group relapsed during the 9-month follow-up period, whereas all 20 patients (100%) in the placebo group developed a relapse ($P<0.05$). Furthermore, all 17 patients who had maintained remission during the study developed relapses within the 4 months after conclusion of active treatment. Fecal concentrations of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus salivarius* organisms increased significantly during treatment with VSL-3, compared with baseline. However, this colonization was transient, demonstrated by the fact that fecal concentrations returned to basal levels 1 month after discontinuation of the probiotic.

The same authors recently presented data suggesting that VSL-3 can be used for prophylaxis of pouchitis after ileostomy closure [21]. Forty consecutive patients who underwent IPAA for ulcerative colitis were randomly assigned to receive either VSL-3 or placebo for 1 year after ileostomy closure. Two of 20 patients (10%) receiving VSL-3 developed acute pouchitis, compared with eight of 20 (40%) patients receiving placebo ($P<0.01$) during the follow-up period.

Several questions regarding probiotic therapy need to be addressed in order to optimize the use of such agents. For example, it is not yet established whether single species probiotic preparations are as effective as preparations like VSL-3, which contains multiple organisms [22]. Also, the optimal dosing and duration of probiotic therapy need to be better defined.

Other therapies

Only two other controlled trials have evaluated therapy for pouchitis using topical agents. A study evaluating 40 patients with chronic active pouchitis found that therapy with bismuth carbomer foam enemas was no more effective than treatment with placebo [23]. Another randomized clinical trial, which evaluated 19 patients with chronic pouchitis, found that relapse rates after a 3-week treatment course were 67% (6/9) in patients receiving glutamine suppositories, compared with 40% (4/10) in those receiving butyrate suppositories ($P<0.05$) [24]. Therefore, there is no difference between glutamine and butyrate suppositories for maintaining remission in patients with chronic pouchitis, and it is unknown whether glutamine and butyrate are equally effective or equally ineffective for this indication.

Postoperative Recurrence of Crohn's Disease

Background

Patients with Crohn's disease who undergo surgical resection have a significant risk of developing recurrent disease. Recurrence rates vary depending on whether endoscopic evidence of disease, clinical symptoms, or need for further surgical resection are the measured endpoints.

Early studies suggested endoscopic recurrence rates ranging between 72% and 93% within 1 year of surgery [25]. A more recent study suggests that the development of endoscopic or radiologic recurrence happens more slowly, with rates of 28% at 1 year and 77% at 3 years [26]. A progression of endoscopic lesions, from scattered aphthous ulcers to severe ulcerations and strictures, is predictive of the subsequent clinical course [25,26].

Reported rates of clinical recurrence after resection of all macroscopically diseased bowel with primary anastomosis are variable but range between 18% and 55% at 5 years and 52% and 76% at 15 years [27]. When surgical recurrence rates are analyzed, approximately 30% of patients will require subsequent resection within 10 years [28].

Specific factors that predispose to postoperative recurrence of Crohn's disease have not been well defined. Patients with initial ileocolic disease were found to be at higher risk for recurrence than were those with disease confined to either the small bowel or the colon in one study, whereas other trials did not demonstrate such a difference [29]. Michelassi *et al.* [29] performed a covariate analysis of several factors

and found that only multiple-site bowel involvement was associated with increased risk for recurrence. Similarly, in another study, patients requiring multiple anastomoses or with evidence of inflammation at the margins were at significantly greater risk for disease recurrence [30].

Perhaps even more intriguing is the suggestion that postoperative recurrences of Crohn's disease can be predicted by the original clinical pattern of disease. D'Haens *et al.* [31] demonstrated that the length of small bowel involved in postoperative recurrence is correlated with the length of diseased intestine prior to surgery. Greenstein *et al.* [32] reported that longer preoperative duration of disease was associated with prolonged recurrence-free survival. These authors subsequently found that the pattern of recurrent disease was consistent with the presurgical subtype. Patients with perforating disease (acute perforation, abscess, or fistula) tended to manifest the same disease pattern when they required further surgery, whereas those with nonperforating disease (obstruction, medical intractability, or hemorrhage) retained a nonperforating clinical pattern at diagnosis of postoperative recurrence. Patients with a "perforating" indication for surgery were also more likely to develop an early postoperative recurrence. Other investigators have reported similar findings [33], although controversy remains with respect to the predictability and consistency of the "perforating" and "nonperforating" classification [34].

Although certain clinical patterns have been described, the underlying pathophysiology of recurrent disease and the reason for its localization to the neoterminal ileum are not well understood. It has long been known that a diverting ileostomy can lead to improvement of symptoms in patients with active Crohn's colitis [35]. In addition, recurrence rates after surgical resection for patients undergoing anastomotic procedures are higher than those for patients requiring end ileostomies. This observation would suggest that fecal stream and bowel continuity play an important causative role in the postoperative recurrence of Crohn's disease [36]. A recent report from Belgium supports this theory [37]. Five patients with a diverting ileostomy proximal to an ileocolic anastomosis were evaluated. These patients had no endoscopic or histologic evidence of anastomotic disease 6 months after surgery, compared with an endoscopic recurrence rate of 71% after 6 months among 75 patients with no diverting ileostomy proximal to an ileocolic anastomosis. Six months after takedown of the diverting ileostomy, all five patients had endoscopic and histologic evidence of recurrent disease. Although these data suggest that fecal stream and reflux of colonic contents into the ileum play a role in the pathogenesis of postoperative Crohn's recurrence, specific antigens or toxins have not been identified.

Smoking is another documented risk factor for postoperative recurrence. Sutherland *et al.* [38] found that surgical recurrence rates were significantly higher in female smokers, compared with female nonsmokers. Similarly, Cottone *et al.* [39] reported that smoking was an independent risk factor for clinical, surgical, and endoscopic Crohn's disease recurrence among patients undergoing surgical resection.

Given the significant recurrence risk following surgical resection in Crohn's disease, identification of preventive measures to reduce these recurrences is important. Various operative techniques have been evaluated, but none has been shown to reduce the risk of recurrence [28]. The role of medical prophylaxis following surgery is unclear, in part because of different diagnostic criteria, drugs, dosing, and duration of treatment and follow-up.

5-Aminosalicylic acid agents

Sulfasalazine has not been shown to be statistically superior to placebo in preventing postoperative Crohn's disease recurrence [40••]. Sulfasalazine delivers the active moiety, 5-aminosalicylic acid (5-ASA), to the colon after bacterial breakdown of an azo-bond linking the 5-ASA to sulfapyridine. Based on this property, little therapeutic effect would be expected in the neoterminal ileum, the most common site of disease recurrence. Theoretically, sulfasalazine would be more beneficial for patients undergoing segmental colonic resection for Crohn's disease.

In contrast to sulfasalazine, agents that deliver mesalamine to specific sites proximal to an ileocolic anastomosis have a theoretical advantage for prevention of postoperative recurrence of Crohn's disease. Seven studies have reported on the use of mesalamine in this setting [40••,41•]. One series reported in abstract form and three published studies have evaluated clinical recurrence rates, two studies have evaluated endoscopic and clinical recurrence rates, and one study has measured endoscopic recurrence rates [40••]. Four of seven studies showed a benefit for mesalamine in reduction of either endoscopic or clinical recurrence in patients with Crohn's disease undergoing surgical resection. Two other studies revealed trends in favor of therapy that were not statistically significant. Although these results suggest that mesalamine does have benefits in reducing the rate of postoperative Crohn's disease recurrence, it is difficult to draw definitive conclusions. However, a recent meta-analysis of the five published studies evaluating clinical recurrence concluded that mesalamine has a statistically significant effect on postoperative recurrence, with a 10% decrease in pooled risk [42,43]. The magnitude of the effect from mesalamine can also be estimated from analyses of the number needed to treat (NNT). The NNT for prevention of endoscopic recurrence is three, whereas the NNT to prevent clinical recurrence in the published trials ranges between four and 10 (Table 2) and was 10 in the meta-analysis.

Metronidazole

Based on data regarding the impact of the fecal stream on postoperative recurrence, the use of postoperative antibiotics appears to be logical. Only a single trial has been published that evaluated the effect of metronidazole on

Table 2. Number needed to treat in mesalamine studies

Study	Countries involved	Measured endpoint	Agent used	Number needed
Lochs *et al.* [41•]	Germany, Austria, Denmark, and Norway	Clinical recurrence	Pentasa (Ferring A/S, Vanlose, Denmark), 4 g/d	5.6*
Caprilli *et al.* [53]	Italy	Endoscopic recurrence Clinical recurrence	Asacol (Bracco SPA, Italy), 2.4 g/d	3.0† 4.3†
Brignola *et al.* [54]	Italy	Endoscopic recurrence	Pentasa (Yamanouchi Pharma SPA, Carugate-Milano, Italy), 3 g/d	3.1‡
McLeod *et al.* [55]	Canada and United States	Clinical recurrence	Rowasa (Solvay, Marietta, GA) Salofalk (Axcan Pharma, Saint-Hilaire, Quebec), 3 g/d	10.0

*Subgroup of patients with small bowel disease.
†Data at 24 months.
‡Recurrence of endoscopically severe lesions (score of 3–4 on 0–4 scale) or abnormal radiology?

postoperative recurrence rates in Crohn's disease [44]. Sixty patients undergoing ileocolonic resection were randomly assigned to treatment with metronidazole (20 mg/kg/d) or to placebo within 1 week of surgery. Treatment was continued for 12 weeks, at which time a colonoscopy was performed. The endpoints of the study were endoscopic recurrence at 12 weeks and at 3 years, as well as clinical recurrence at 3 years. After 12 weeks, colonoscopy revealed recurrence in 52% of patients on metronidazole, compared with 75% on placebo (P=0.09). Endoscopic lesions were also less severe in the metronidazole group (P=0.02). By 3 years, however, the number of endoscopic recurrences was equal in the two groups (78% for metronidazole vs 82% for placebo). A significant effect of metronidazole on clinical recurrence was observed at 1 year (4% for metronidazole vs 25% for placebo; P=0.046). A trend toward lower clinical recurrence at 2 and 3 years after metronidazole was observed, but these differences were not statistically significant. The positive results were offset by prominent side effects related to the higher doses of metronidazole. Additional trials are needed to assess lower, more tolerable doses of metronidazole administered for longer periods after resection, as well as to evaluate alternative antibiotics.

Six-Mercaptopurine and Azathioprine

Six-Mercaptopurine (6-MP) and azathioprine have been shown to maintain medically induced remissions in Crohn's disease [45]. One could thus anticipate a similar benefit for patients with surgically induced remission.

The only controlled trial involving a large number of patients was recently reported in abstract form [46]. The authors of this 2-year, five-center trial involving 131 patients studied the effects of 6-MP (50 mg/d) compared with mesalamine (3 g/d) or placebo on the rate of postoperative clinical, endoscopic, and radiographic relapse. Life-table analyses demonstrated that 6-MP was statistically superior to placebo in decreasing clinical relapse and superior to both mesalamine and placebo in decreasing significant endoscopic relapse.

In addition, data from two uncontrolled studies suggest a potential benefit of 6-MP or azathioprine in the prevention of postoperative recurrence [47,48]. Lemann *et al.* [47] reported in an abstract on 38 patients who received azathioprine (2 mg/kg/d) either before (n=19) or within 3 months (n=19) of surgical resection and who were followed for a median of 16 months. Clinical recurrence rates were 9% at 1 year and 19% at 2 years, whereas endoscopic recurrence rates were 21% at 1 year and 31% at 2 years. Although there was no control group in this study, these rates are lower than those reported for historical controls. Similarly, Korelitz *et al.* [48] reported that nine of 10 patients who were placed on 6-MP (no mean dose noted) after a second resection for Crohn's disease had not developed clinical recurrence after a mean follow-up of 41 months.

These preliminary results are encouraging, but additional controlled trials are needed to fully assess the dose response and long-term safety of 6-MP or azathioprine in the prevention of postoperative Crohn's disease recurrence.

Corticosteroids

Corticosteroids have not shown a maintenance benefit in medical therapy for Crohn's disease [49]. In contrast to conventional steroids, budesonide has the potential advantages of potent topical anti-inflammatory activity due to high steroid receptor affinity, low systemic effects due to rapid first-pass hepatic metabolism, and ability to develop pharmacologic targeting to distal small bowel or colonic sites for drug delivery [50].

A recently published trial from Europe described 129 patients randomly assigned either to treatment with a controlled-ileal-release budesonide formulation (6 mg/d) or to placebo within 14 days of surgical resection [51]. No differences in endoscopic recurrence rates were found at 3 or 12 months. However, in subgroup analysis, a trend was shown toward decreased recurrence with budesonide for patients who had undergone surgery for "high disease activity." Similarly, a German group found no significant difference in recurrence (endoscopic and/or clinical) at 1

year following surgery when 83 patients were randomly assigned to receive either delayed-release budesonide (3 mg/d) or placebo within 2 weeks of surgery [52]. Further study of this agent is needed to better define its effect in decreasing postoperative recurrence.

Conclusions

The main long-term complication of IPAA for patients with ulcerative colitis is pouchitis. Pouchitis is a poorly understood inflammatory process manifested by symptoms of increased stool frequency, fecal urgency, and abdominal cramping. In clinical practice, pouchitis is often diagnosed based on symptoms alone and empirically treated with antibiotics such as metronidazole. However, a recent study demonstrated that symptom assessment alone is not reliable for accurate diagnosis of pouchitis. Only a small number of controlled trials have evaluated therapy for pouchitis, but data are available to support the use of metronidazole in active pouchitis and of VSL-3 as maintenance therapy for acute relapsing pouchitis.

Patients with Crohn's disease who undergo surgical resection have a significant risk of developing recurrent disease, but medical therapy can help decrease this risk. It is important to identify patients who may be at increased risk for postoperative recurrence in order to maximize and better direct the use of medical therapy and possible preventive measures in this setting. Factors that may play an important role include disease type and location, multiple sites of disease, surgical procedure, and tobacco use. Mesalamine has been shown to lower postoperative recurrence rates of Crohn's disease and should be considered for postoperative prophylaxis. Metronidazole and 6-MP/azathioprine appear to be beneficial in post operative therapy, but additional controlled studies are required to better define the efficacy and dose response of these agents. Recommendations for individual patients should be based on the patient's willingness to take medications on a prophylactic basis and on the potential costs and side effects of treatment.

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Exhibit H

CARFAX

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Pouchitis

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POUCHITIS is a major long-term complication of the continent ileostomy as well as the ileoanal pouch anastomosis. When diagnosed on the basis of clinical, endoscopic and histologic features, this syndrome has been demonstrated almost exclusively in patients with ulcerative colitis. The clinical course, the endoscopic findings and the histologic abnormalities resemble those of ulcerative colitis. The association with extra-intestinal manifestations further supports the hypothesis that pouchitis represents ulcerative colitis in the small bowel. All ileal reservoirs show bacterial overgrowth, especially of anaerobes. As a response to this altered intraluminal environment chronic inflammation and incomplete colonic metaplasia occur. The efficiency of metronidazole does suggest that bacteriological factors play an important role in the pathogenesis of pouchitis.

Key words: ulcerative colitis, ileostomy, ileoanal anastomosis, pouchitis, metronidazole

Introduction

In the past, a permanent Brooke ileostomy was inevitable for patients requiring a proctocolectomy for either ulcerative colitis or familial adenomatous polyposis. During the past two decades, the continent ileostomy, devised by Kock, and the ileoanal anastomosis, introduced by Parks and Utsunomiya, have evolved into attractive alternatives. Both procedures have the advantage of removing all diseased mucosa while avoiding a conventional and incontinent ileostomy. The construction of an ileal reservoir, however, frequently results in mucosal alterations. Although most of these alterations remain subclinical, some patients will develop a clinical syndrome known as pouchitis. Although it has been suggested that faecal stasis with subsequent alterations in bacterial flora might be important in the pathogenesis of pouchitis, the exact role of intestinal microflora remains controversial. Therefore it might be worthwile to review the current concepts with regard to pathogenesis and aetiology of pouchitis and to analyse the different treatment modalities.

History

In the 1940s and early 1950s it became apparent that mucosal inflammation immediately proximal to the ileostomy was a not uncommon complication after colectomy for ulcerative colitis.[1] This prestomal ileitis resulted occasionally in perforation of the diseased small bowel as described by Crandon *et al.* in 1944.[2] Initially this complication was felt to be related to preoperative 'backwash' ileitis.[1–4] In 1956 Counsell reported successful treatment of prestomal ileitis by stomal dilatation and lavage with a catheter. Since then it became widely accepted that prestomal ileitis was secondary to chronic ileostomy obstruction.[5] In 1976 Kock reported mucosal inflammation in 14 out of 164 patients in whom a continent ileostomy was constructed. The inflammatory changes in the reservoir occurred soon after pouch construction or as late as several years and were associated with an increase in ileostomy output and a foul-smelling bloody effluent. Other symptoms such as nausea, vomiting and fever were also present. All patients had been successfully treated by catheter drainage and sulphasalazine.[6] Kock suggested that this mucosal inflammation was due to fecal stasis and overgrowth of anaerobic bacteria and advocated the term pouchitis to describe this nonspecific ileitis. This syndrome, which also occurs in pelvic reservoirs after ileoanal anastomosis, has been described variably as stagnant loop syndrome[7] or mucosal enteritis.[8,9]

Incidence

The reported incidence of pouchitis following restorative proctocolectomy varies considerably from 10% to 50% (Table 1). This discrepancy is to a great extent due to the variability in definition, the different numbers of patients investigated and the different length of follow-up. Furthermore, in most series

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complete details of endoscopic and histologic features have been infrequently described. Similar figures have been documented in consecutive series of patients with a continent ileostomy. Hultén *et al.*[33] reported that the cumulative probability of developing a first attack of pouchitis over a 10-year period is about 35% of patients with a Kock-pouch. Life table analysis of data derived from a register of all patients who have undergone ileoanal anastomosis at the Mayo Clinic revealed a cumulative risk of pouchitis of 31% for patients with ulcerative colitis.[21] Although pouchitis occurs both early and late following reservoir construction, most patients develop their first episode within 2 years postoperatively.[22,28] Approximately half of the patients have only one single episode, whereas the others present two or more episodes.[22,24] Rauh *et al.*[24] reported a preponderance of indeterminate colitis in patients with recurrent episodes of pouchitis. Although pouchitis has been reported to occur before ileostomy closure, this complication is seen predominantly after ileostomy closure.[28] Another intriguing observation is that pouchitis appears confined to patients operated on for ulcerative colitis, whether the pouch is placed in the pelvis or constructed as a continent ileostomy.[34] However, in 1990 Kmiot *et al.*[35] reported a fully documented case of pouchitis in a patient following ileal reservoir construction for familial adenomatous polyposis. A similar case has been described in 1991 by Rauh *et al.*[24] Reviewing their patients operated on for familial adenomatous polyposis, Lohmuller *et al.*[21] found a cumulative risk of pouchitis of 6% However, in this study pouchitis was defined as present if patients had abdominal cramping, watery diarrhoea, urgency, incontinence, malaise and fever, without endoscopic evaluation and histopathologic confirmation. Despite these and other anecdotal reports it is widely accepted that pouchitis is confined to patients operated on for ulcerative colitis.

Table 1. Incidence of pouchitis following restorative proctocolectomy

Author	Year	Pouchitis (%)
Fonkalsrud[10]	1984	44
Nicholls *et al.*[11]	1985	11
Schoetz *et al.*[12]	1986	7
Becker and Raymond[13]	1986	18
O'Connell *et al.*[14]	1986	30
Gustavsson *et al.*[15]	1987	15
Pemberton *et al.*[16]	1987	14
Fleshman *et al.*[17]	1988	16
Pescatori *et al.*[18]	1988	14
Everett[19]	1989	27
Oresland *et al.*[20]	1989	30
Lohmuller *et al.*[21]	1990	29
Wexner *et al.*[22]	1990	27
DiFebo *et al.*[23]	1990	13
Rauh *et al.*[24]	1991	14
Santavirta *et al.*[25]	1991	30
De Silva *et al.*[26]	1991	21
McMullen *et al.*[27]	1991	15
Fozard and Pemberton[28]	1992	31
Clausen *et al.*[29]	1992	18
Gemlo *et al.*[30]	1992	31
Luukkonen *et al.*[31]	1994	23
Ståhlberg *et al.*[32]	1996	51

Diagnostic Criteria

Pouchitis has been defined using various criteria. Some authors have favoured a diagnosis based on clinical symptoms, whereas others recommended the use of endoscopic or histologic features. Because different diagnostic criteria have been adopted, it is difficult to interpret the reported data related to pouchitis. Taking this into account, it is obvious that there is a need for a gold standard in diagnosis. Recently, it has been advocated that an unequivocal diagnosis should be based on a diagnostic triad, consisting of the following components: clinical symptoms, endoscopic features of acute inflammation and histological evidence of a prominent polymorphonuclear cell exudate.[34]

Clinical symptoms

Watery and sometimes bloody diarrhoea is the major clinical symptom of pouchitis. The increased frequency of stools may be associated with abdominal discomfort, urgency, incontinence and even dehydration. Some patients also have fever and malaise. It has become apparent that pouchitis has the ability to evoke arthritis, skin lesions and eye problems, resembling the extra-intestinal manifestations of inflammatory bowel disease. Lohmuller *et al.*[21] showed that patients with preoperative extra-intestinal manifestations had significant higher rates of pouchitis than did patients without these manifestations (39% vs. 26%). They also described patients in whom extra-intestinal manifestations only recurred when pouchitis occurred and abated when pouchitis was treated.[21] This relationship is one of the intriguing findings suggesting that pouchitis is likely associated with the underlying pathophysiologic mechanism involved in ulcerative colitis.

Endoscopic features

As soon as faecal material enters the pouch, its endoscopic aspect begins to change. The mucosa becomes slightly swollen and somewhat redder in appearance.[36] These mild inflammatory changes, however, seem to be present in only a few cases. DiFebo *et al.*[23] found normal mucosa in 33 out of 41 asymptomatic patients with a pelvic reservoir, whereas endoscopy revealed focal lesions including oedema, petechiae and single ulcers in eight patients without clinical symptoms of pouchitis. Endoscopic

criteria for pouchitis are well known indicators of an acute non-specific inflammation: granularity, oedema, erythema, friability, petechiae, hypersecretion and multiple superficial erosive defects. Although these changes may be focal, they frequently affect all the mucosa, extending sometimes into the afferent limb of the ileum above. In the majority of cases the endoscopic features of pouchitis mimic those of ulcerative colitis. In some patients, however, endoscopic aspects resembles pseudomembranous enteritis, whereas in other patients ulcers are observed similar to those seen in Crohn's disease.[23] The degree of macroscopic inflammation seems to be related to the frequency of defecation as well as to the histological grade of acute inflammation.[37]

Histologic criteria

Several studies have shown that ileal pouch mucosa undergoes morphological changes as soon as faecal material enters the pouch. In the majority of patients mucosal biopsy specimens reveal a chronic inflammatory infiltrate in the lamina propria, including lymphocytes, plasma cells, eosinophils and histiocytes. Such an infiltrate, associated with some degree of villous atrophy and crypt hyperplasia, was found in 87% of the reservoirs, studied by Shepherd *et al.*[38] Patients with ulcerative colitis did not show a significant difference in chronic inflammatory score compared with those operated on for familial adenomatous polyposis. The histopathological appearance of chronic inflammation combined with villous atrophy resembles that of inactive ulcerative colitis. It has been noted that in patients with a conventional ileostomy the normal villous architecture of the prestomal mucosa is preserved, despite the presence of chronic inflammatory changes.[39] This finding indicates that flattening of the villi and crypt hyperplasia is more likely to be induced after construction of an ileal reservoir than after the creation of a conventional ileostomy. It has been suggested that these morphological changes, which are irrespective of the original diagnosis, reflect an adaptive response to the new luminal environment. The change from villous structure of small bowel to a glandular morphology of colon is sometimes so pronounced that biopsy specimens are indistinguishable from normal colon on routine histologic examination. Initially this metaplasia has been defined by means of the histological changes, such as villous atrophy, crypt hyperplasia and increased numbers of Goblet cells and lysozyme containing Paneth's cells. Recent histochemical studies, however, have shown that in 50% of the cases colonic metaplasia is also characterized by a change from small intestinal sialomucin to colorectal sulphamucin.[38,40] Despite this metaplasia, pouch mucosa retains small bowel characteristics, supported by the finding of sucrase-isomaltase activity in pouch specimens.[40] Furthermore, it has been shown that no alteration occurs in endocrine cell population.[41] In pouchitis the mucosa shows a dense acute inflammatory cell infiltrate, consisting of polymorphic granulocytes, associated with crypt abscesses and ulcerations. Frequently the villous atrophy becomes more extensive and subtotal. The histological grade of acute inflammation is significantly related to the clinical symptoms.[37] The histologic findings in pouchitis are very similar to those seen in acute ulcerative colitis.

Pathogenesis

Bacterial overgrowth

Faecal stasis with bacterial overgrowth has been considered a major contributing factor in the pathogenesis of pouchitis. Ileal reservoirs are colonized with large numbers of bacteria that outnumber the flora of the normal terminal ileum.[14,25,39,42–45] In ileal reservoirs, without signs of pouchitis, the microflora closely resembles the flora of the large bowel. This is mainly due to the large numbers of anaerobes (especially Bacteroides and Bifidobacteria), resulting in a greater ratio of anaerobes to aerobes.[25,42–46] In only one study bacterial counts in ileal reservoirs were identical with normal stool values.[47] Other studies, however, revealed that the microflora holds an intermediate position between ileostomy effluent and normal faeces.[42,46,48] It has been suggested that incomplete emptying of the pouch, which is associated with stasis of ileal contents, would result in an increase in the number of anaerobic bacteria. Comparing S and W reservoirs Sagar *et al.*[49] found a reduced efficiency of evacuation in S reservoirs. The effluent of these reservoirs had a significantly greater number of Bacteroides. In another study, however, bacterial overgrowth with an increased number of anaerobes was found in all pouches, irrespective of the efficiency of evacuation.[14] In both studies no correlation was found between the efficiency of evacuation and the grade of mucosal inflammation. Similar findings have been reported by others.[39,50] Therefore, it seems likely that exposure to the faecal stream, rather than the amount of stasis, is the 'threshold' factor for the development of mucosal changes found in ileal reservoirs. The increased numbers of bacteria appear responsible for the increased crypt cell production rate and villous atrophy observed in the pouch mucosa soon after the construction of the reservoir.[43] Nasmyth *et al.*[45] found a significant correlation between the number of isolated Bacteroides and the grade of villous atrophy. The greater the number of Bacteroides the more severe was the villous atrophy. Conversely, the higher the concentration of feacal butyrate the less severe was the villous atrophy.[45] Both findings appear to be contradictory, because volatile fatty acids, such as

butyrate, are the product of anaerobic bacterial fermentation of intraluminal carbohydrate. However, very few species of Bacteroides produce butyrate and it might be speculated that *in vivo* butyrate suppresses the growth of Bacteroides. It is yet not clear whether the grade of chronic inflammation correlates with the number of bacteria isolated. In two studies the score for chronic inflammation was correlated to the number of anaerobes.[25,51] However, other investigators could not demonstrate such a consistent correlation between bacterial counts and chronic inflammation.[45,52] The prompt response in some patients with clinical pouchitis to metronidazole suggests the possibility that overgrowth of anaerobes may be important. However, there is a great deal of controversy concerning the correlation between anaerobes and pouchitis. Several studies failed to show a quantitative or qualitative difference between the microbial findings in patients with and without pouchitis.[14,39,44,53] A recent study, conducted at our own institution, also failed to show significant differences in the total numbers of bacteria when pouch effluent from controls and patients was compared. However, patients with pouchitis had a different composition of the flora. Several anaerobes, such as bifidobacteria and anaerobic lactobacilli, disappeared in favour of aerobes. This was reflected in the ratio anaerobes to aerobes: patients without pouchitis harboured more than hundred times more anaerobes than aerobes. Patients with pouchitis had only two times more anaerobes.[54] These observations have been confirmed by Onderdonk *et al.*[55] who cultered significantly more aerobes from tissue biopsy samples from patients with pouchitis than from control patients. Our study also revealed that the flora of patients with pouchitis is rather unstable. We cultured several species that were not found in controls, such as fungi, Bacillus species and Candida species. Furthermore, *Clostridium perfringens* was detected in nearly every pouchitis, sometimes in very high numbers.[54] A selective increase of *Clostridium perfringens* has also been documented by Brandi and coworkers.[56] The exact role of C perfringens in the pathogenesis of pouchitis is still unknown.

Mucosal ischaemia

It has been suggested that transient ischaemia and subsequent reperfusion may be an aetiological factor in the pathogenesis of pouchitis. It is well known that the vessels supplying the terminal ileum are often under tension when the ileoanal anastomosis is completed. Frequently, these vessels must be divided to provide adequate length for performing the anastomosis. Using fluorescein flowmetry and laser Doppler flowmetry it has been shown that mucosal bloodflow in pelvic reservoirs is significantly reduced compared with the mucosal bloodflow in conventional ileostomies.[57,58] Sakaguchi *et al.* have reported that in patients with pouchitis the mucosal bloodflow was less than in healthy reservoirs.[58] In ischaemic tissues, xanthine dehydrogenase is converted to xanthine oxidase. During reperfusion this enzyme catalyses a reaction resulting in the liberation of oxygen-derived free radicals, which can be prevented by the administration of allopurinol. To investigate the role of this xanthine oxydase inhibitor Levin *et al.* conducted a study in patients with pouchitis. They found a benefical effect of allopurinol in 50% of the patients, either with acute or chronic pouchitis.[53] The results of this preliminary study are consistent with a role for mucosal ischaemia in the aetiology of pouchitis.

Short-chain fatty acids

Short-chain fatty acids (SCFAs) are the product of anaerobic bacterial fermentation of dietary fibres. They are the preferred energy substrates for colonocytes and have a trophic effect on the large bowel mucosa. It has been suggested that these SCFAs are also an important energy source for the pouch epithelium, which can undergo colonic metaplasia. Moreover, it has been shown that SCFAs are able to suppress enteropathic bacteria that produce toxic metabolites, which in turn may cause mucosal inflammation.[59] In view of the increased numbers of anaerobes, increased production of SCFAs might be expected in ileal reservoirs. Nasmyth *et al.*[45] demonstrated that the concentration of SCFAs in the effluent from normal pouches exceeds that from ileostomies. However, no significant difference was found between the SCFA-concentration in faecal specimens from pouch patients and normal subjects. The only difference between the effluent from pouches and that from normal subjects was a higher concentration of acetate in the effluent from the pouches.[45] In contrast with this finding, Ambroze *et al.*[60] reported lower concentrations of SCFAs in pouch effluent compared with normal stool. In a preliminary report Wischmeyer *et al.*[61] described reduced concentrations of SCFAs in patients with pouchitis compared with patients without pouchitis. Recently, this finding was confirmed by others.[29] It seems likely that the lower concentrations of SCFAs are due to the reduced numbers of anaerobes. Whether the reduced concentrations of SCFAs are the result rather than the cause of pouchitis has not been determined. The effect of local application of SCFAs on pouchitis has been studied by DeSilva *et al.*[62] Two patients with severe pouchitis that was resistant to treatment with metronidazole, 5-amino salicylic acid and corticosteroids, received 60 ml of a SCFA solution twice daily. Treatment was discontinued when deterioration was seen in both patients after 14 and 28 days respectively. Based on these results it seems unlikely that low concentrations of

SCFAs are important in the pathogenesis of pouchitis.[62]

Bile acids

It has been suggested that the bacterial overgrowth in ileal reservoirs might result in an increased bacterial deconjugation of bile acids. It is well known that the bacteria in the terminal ileum are able to hydrolyse the conjugated bile acids and to dehydroxylate the bile acids to secondary bile acids. It has been shown that desoxycholic acid (a secondary bile acid) causes a progressive increase in water and salt permeability followed by cell death in the rat colon.[63] Could secondary and deconjugated bile acids cause pouchitis? In one study, comparing patients with and without pouchitis, the concentrations of both total conjugated bile acids and tauroconjugated bile acids were found to be lower in pouchitis patients, which suggests an increased bacterial deconjugation in pouchitis.[59] In another study it has been shown that ileal pouch dialysate is cytotoxic to intestinal epithelial cell lines. This effect was inhibited by cholestyramine, which suggests that a bile acid may be the cytotoxic factor.[64]

Recurrence of ulcerative colitis

One of the most intriguing aspects of pouchitis is the observation that this complication occurs almost exclusively in patients who undergo colectomy for ulcerative colitis. Based on this finding, it has been suggested that ulcerative colitis and pouchitis share the same aetiology. The observation that some patients with inflamed reservoirs experience extraintestinal manifestations resembling those occurring in ulcerative colitis supports this theory. Many studies have confirmed that the pouch mucosa undergoes morphological changes and acquires characteristics resembling those of colonic mucosa. This colonic metaplasia seems to be a nonspecific adaptive response to the new luminal environment that favours the development of an ulcerative colitis-like condition.[65] Exposure to the faecal stream is probably the initiating event that allows the onset of inflammatory changes.[66] It has been shown that colonic mucin glycoproteins are altered in patients with ulcerative colitis.[67] It could be possible that the aberrated glycoproteins are more susceptible for bacterial enzymatic degradation, making the mucus barrier less resistant to toxins. The findings that pouch mucin resembles colonic mucin is therefore an important one. In recent years increasing numbers of data further support the hypothesis that pouchitis represents recurrent ulcerative colitis. In a study aimed to characterize the mucosal cellular infiltrate in ileal reservoirs, de Silva *et al.*[68] found increased RDF9+ macrophage subpopulations in pouchitis. This finding suggests that the effector mechanisms triggering pouchitis are similar to those in ulcerative colitis. In another study the production of eicosanoids, arachidonic acid and interleukin-1β was found to be elevated in inflamed reservoirs, indicating that in pouchitis the same inflammatory mediators are involved as in ulcerative colitis.[69] An increased expression of cell adhesion molecules (E selection and intercellular adhesion molecule-1) has been demonstrated in pouchitis, similar to that reported in ulcerative colitis.[70] Like ulcerative colitis, pouchitis is associated with an increased production of platelet-activating factor, indicating that both disorders share the same aetiology.[66] Merrett *et al.* reported fewer episodes of pouchitis in smokers than in non-smokers.[71] Such a 'protective' influence has previously been described in smokers with ulcerative colitis. All these data suggest that ulcerative colitis can occur in the small intestine on the condition that the luminal environment acquires certain colonic characteristics. Bacterial overgrowth is probably the initiating event in this process of colonic metaplasia.

Treatment

Numerous anecdotal reports have shown that pouchitis is responsive to antibacterial therapy with metronidazole. According to Fozard and Pemberton the majority of patients respond rapidly to a short course of treatment.[28] In their series only 3% of the patients were refractory to this therapy or had severe side effects. O'Connell reported that all his patients with pouchitis obtained prompt relief of symptoms.[14] Comparing pouchitis with and without mucosal ulceration, Zuccaro *et al.*[72] observed a therapeutic effect of metronidazole in 20% and 78% respectively. This finding indicates that antibacterial treatment is probably less effective than previously reported. Based on the observation that some patients do not respond to metronidazole, it has been suggested that there are at least two forms of pouchitis: a bacteriological one that responds to metronidazole and one that requires other medication. The effectiveness of metronidazole can only be assessed in a controlled trial, which is also necessary for proper recommendations regarding dosage schedules and duration of treatment. The observation that clinical symptoms are often resolved with a short course of metronidazole supports a bacteriological basis of pouchitis. However, the actual mechanism of action of metronidazole is still uncertain. Levin *et al.* suggested that metronidazole affects pouchitis not by an antibacterial action, but rather by its capacity to remove oxygen radicals.[53] Other workers raised the possibility that metronidazole has a therapeutic effect because of its immunosuppressive activity.[73] This is of interest as metronidazole does not appear to have a role in the treatment

of ulcerative colitis.[74] It is obvious that the mechanism of action of metronidazole can only be elucidated in a study comparing pouch microflora before and after treatment with metronidazole, whether the therapy is successful or not. Recent studies suggest that pouchitis is a chronic relapsing complication with reported recurrence rates varying between 50% and 80%[21,23,31,75] It appears that an increasing number of patients will require intermittent or maintenance therapy. The question is whether metronidazole is suitable for that purpose or not, particularly in the light of the potential for peripheral neuropathy and other side effects. Patients who are refractory to treatment with metronidazole might obtain relief of symptoms after the administration of enemas containing salicylic acid derivates.[76] Even the use of steroids has been advocated in the treatment of persistent pouchitis. However, continuous administration of steroids with the intention of saving a sick pouch is questionable. Despite their suggested role in the pathogenesis of pouchitis, short-chain fatty acids appear to be of no value in the treatment of pouchitis.[62] Recently it has been shown that oxygen-derived as well as leukocyte-derived free radicals are involved in the pathogenesis of ulcerative colitis. Levin demonstrated that allopurinol, a scavenger directed against oxygen-derived free radicals, induced a remission in 50% of the patients.[53] The value of other scavengers, directed against leukocyte-derived free radicals, such as superoxide dismutase, is still unknown.

There is growing evidence that the pouch flora is very susceptible to influences from outside, such as dietary variation; stress and bacterial contamination. This instability may lead to microbial imbalance, which might be a major contributing factor in the pathogenesis of pouchitis.[54] Based on this assumption it might be worthwhile to bring about a stable pouch flora. This might be realized by oral ingestion of lactobacilli, which has been proved to be successful in the treatment of intestinal infections and antibiotic associated diarrhoea.[54]

Summary

It might be possible that bacterial enzymes, such as glycosidases, degrade the protecting mucus, which may become more permeable to toxic bacterial metabolites and host-derived proteolytic enzymes, affecting the integrity of the mucosa. As a result bacterial antigens may cross the mucosal barrier. This translocation of bacterial antigens probably triggers a cascade of inflammatory events. Only in patients with ulcerative colitis these inflammatory events finally result in clinical pouchitis. Ulcerative colitis is a condition with the potential of neoplastic change in the large intestine. If pouchitis represents recurrent ulcerative colitis, then the pouch epithelium might be prone to malignant transformation. Although the colonic metaplasia is not complete, the reservoir mucosa shows hyperproliferation both in patients with pouchitis and in those without this syndrome.[65] Recently Löfberg *et al.*[77] reported dysplasia and DNA aneuploidy in the pelvic pouch of a patient with ulcerative colitis. Stern *et al.*[78] described the development of a carcinoma in an ileal reservoir of a colitis patient. Based on these findings long-term endoscopic surveillance of the reservoir mucosa has been recommended.

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Exhibit I

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Diagnosis and Management of Pouchitis

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The disease pouchitis was first reported by Kock[1] in 1977 as an inflammatory condition of the continent ileal reservoir (Kock pouch) in patients who had undergone proctocolectomy. The Kock pouch was later replaced by the ileal pouch anal anastomosis (IPAA, also known as the ileoanal pouch) which was independently described by Parks and Utsunomiya[2,3] in 1980. The ileoanal pouch is now the surgical option of choice in patients with familial adenomatous polyposis (FAP) and ulcerative colitis (UC) with either dysplasia or disease refractory to medical therapy. Pouchitis is the most common long-term complication of IPAA in UC.[4] This review discusses the diagnostic criteria, cause, and management of acute and chronic pouchitis.

Definition

The variation in the reported frequency of pouchitis at different centers and at the same center at different points in time is a reflection of the lack of uniform classification and diagnostic criteria. The definition of pouchitis has evolved to encompass clinical, endoscopic, and histologic criteria. A sensitive but nonspecific designation developed by the Mayo Clinic in 1987 defined pouchitis as a clinical syndrome of watery, frequent, at times bloody stool accompanied by urgency, incontinence, abdominal cramps, malaise, and fever. The symptoms must be present for at least 2 days and should be relieved within 48 hours by metronidazole therapy.[4] A more specific diagnostic criteria proposed by the St. Marks Hospital defined pouchitis as a triad of diarrhea ($\geq$6 stools/day), endoscopic findings ($\geq$ 4 findings of edema, granularity, friability, loss of vascular pattern, mucosal hemorrhage, or ulceration), and a minimum grade of 4 in a 6-point histopathologic index (polymorphonuclear leukocyte infiltration and percent ulceration per low-power field).[5]

The Pouchitis Disease Activity Index (PDAI) was developed in 1994, incorporating the Mayo Clinic definition and the St. Marks pouchitis triad and histopathologic index.[6] The PDAI attempted to provide a standardized definition of pouchitis based on clinical, endoscopic, and histologic markers (Table 1), with pouchitis defined as a score greater than or equal to 7 points. The specificity and sensitivity of diagnosis was increased by defining the disease as a continuum from mild to severe pouchitis with symptoms individualized to the norms of each patient. The operational use of the PDAI has evolved such that active pouchitis is defined as a PDAI score greater than or equal to 7 points in a patient with a definite diagnosis of pouchitis, whereas a PDAI score greater than or equal to 7 points in a patient with a history of a definite diagnosis of pouchitis indicates that the pouchitis is in remission.

In 2001, Heuschen described the Heidelberg Pouchitis Activity Score (PAS),[7] which again attempted to provide a common definition of pouchitis (Table 2). The PAS and PDAI are very similar with the major exception of the inclusion within the former of chronic inflammation as a variable in the histopathology category, the exclusion of fever among the clinical symptoms, and minor variations in the endoscopic score. Heuschen then applied both the PAS and PDAI to 41 patients over 103 outpatient visits and compared them with the gold standard of a physician and surgeon's independent diagnosis of pouchitis.[8] The clinicians diagnosed pouchitis in 24.3% of patients, the PAS in 35.9%, and the PDAI in 17.5%. When compared to the clinician, the PAS had a sensitivity and specificity of 84% and 79.5%, respectively, while the PDAI had a sensitivity and specificity of 60% and 96.2%, respectively. In patients with and without pouchitis, there was no significant difference in the clinical symptoms score in the PAS or the PDAI, but there was a difference in the total endoscopic score and the total histologic score. In addition, although the endoscopic and histologic examinations correlated in both the PAS and the PDAI, there was no correlation

Abbreviations used in this paper: EIM, extraintestinal manifestations; FAP, familial adenomatous polyposis; IL, interleukin; IPAA, ileal pouch anal anastomosis; pANCA, serum antineutrophil cytoplasmic antibody-perinuclear staining pattern; PAS, Pouchitis Activity Score; PDAI, Pouchitis Disease Activity Index; PSC, primary sclerosing cholangitis; QOL, quality of life; SCFA, small chain fatty acids.

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between clinical and endoscopic or clinical and histologic findings in either scoring system.

Overall, the PAS seems to overestimate pouchitis by 11% and the PDAI seems to underestimate pouchitis by 18% when compared with the gold standard of the clinician's assessment. Both the PDAI and the Heidelberg PAS need to be revalidated to determine the scores required to define symptomatic remission and global remission, and to determine the minimum clinically significant difference in the scores needed to define symptomatic improvement and global improvement.

Once a diagnosis of pouchitis is made, it can be further classified.[9] The activity of pouchitis is stratified as remission (no active pouchitis), mild to moderately active (increased stool frequency, urgency, infrequent incontinence), or severely active (hospitalization for dehydration, frequent incontinence). The duration of pouchitis is defined as acute ($\leq$ 4 weeks) or chronic ($>$ 4 weeks) and the pattern of pouchitis is classified as infrequent (1 or 2 acute episodes), relapsing ($\geq$ 3 acute episodes), or continuous. Finally, the response to medical therapy is labeled as treatment-responsive or treatment-refractory with the medications for either case specified.

Table 1. The Pouchitis Disease Activity Index

Clinical criteria	Score
Stool frequency	
Usual postoperative stool frequency	0
1–2 stools/day > postoperative usual	1
3 or more stools/day > postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency/abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature > 100[5] Farenheit)	
Absent	0
Present	1
Endoscopic criteria	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucus exudates	1
Ulceration	1
Acute histologic criteria	
Polymorph infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field (average)	
<25%	1
≥25% ≤ 50%	2
>50%	3

Pouchitis is defined as a total PDAI score ≥ 7 points.
Adapted with permission from: Sandborn WJ, Tremaine WJ, Batts KP, Pemberton JH, Phillips SF. Pouchitis following ileal pouch-anal anastomosis: a pouchitis disease activity index. Mayo Clin Proc 1994;69: 409–415.

Table 2. The Heidelberg Pouchitis Activity Score: Maximum 36 Points

Clinic	Score		Score
1. Stool frequency/ 24 hours		2. Fecal urgency	
< 8	0	absent	0
8–10	2	present	3
11–13	4		
>13	6		
3. Rectal bleeding			
absent	0		
present	3		
			Max. 12

Endoscopy	Score		Score
1. Edema		2. Granularity	
absent	0	absent	0
present	1	present	1
3. Friability		4. Erythema	
absent	0	absent	0
mild	1	mild	2
severe	2	severe	3
5. Flattening of mucosal surface		6. Ulcerations/erosions	
absent	0	absent	0
present	2	mild	2
		severe	3
			Max. 12

Histology	Score		Score
1. Acute histologic inflammation		2. Chronic histologic inflammation	
Polymorphonuclear leukocyte infiltration		Mononuclear leukocyte infiltration	
absent	0	absent	0
discrete and patchy (largely confined to surface epithelium)	1	mild and patchy	1
moderate with (±) crypt abscesses or cryptitis	2	moderate	2
extensive with (±) crypt abscesses or cryptitis	3	extensive	3
Ulcerations/erosions		Villous atrophy	
absent	0	absent	0
mild and superficial	1	minimal	1
moderate	2	partial	2
extensive	3	subtotal/total	3
			Max. 12

Reprinted with permission from Heuschen et al. Dis Colon Rectum 2001;44:487–499.

Diagnostic Tests

The key point of both the PDAI and the PAS is that endoscopic and histopathologic evaluation is required to make the diagnosis of pouchitis. This finding was corroborated by Shen[10] in a study applying the PDAI to the evaluation of 46 patients who had ileal pouches.

Forty-eight percent of patients were given a diagnosis of pouchitis based on a PDAI score of ≥7. No correlation was found between the symptom, endoscopy, and histology scores. Patients who had low clinical scores, but a PDAI of ≥ 7 decreased their PDAI by ≥ 3 points after 2 weeks of antibiotic therapy. The mean reductions in the total PDAI score, symptom, endoscopy, and histology scores were all significantly lower than before treatment. Conversely, 25% of patients who had clinical symptoms of pouchitis who did not meet the PDAI criteria for pouchitis did not respond symptomatically to empiric antibiotic therapy in the past. This latter group of patients can be classified as having irritable pouch syndrome.[11]

On endoscopy, the neoterminal ileum above the pouch should be normal; inflammation and ulceration here indicates Crohn's disease. Inflammation of the pouch mucosa with granularity, edema, mucosal hemorrhage, contact bleeding, and superficial ulcers can be present with varying degrees of severity.[12] Inflammation can be uniform throughout the pouch or more severe in the distal pouch.[13] Histopathologic findings in pouchitis include acute and chronic inflammatory cell infiltration, ulceration, and villous atrophy with crypt abscesses and hyperplasia.[14]

If pouchitis is refractory to medical therapy or has atypical components, further diagnostic tests should be performed to exclude alternate diagnoses. Infectious etiologies should be ruled out by stool sampling and pouch biopsy. Multiple cases in the literature document cytomegalovirus of the pouch in patients who had refractory pouchitis. Treatment with ganciclovir led to resolution of symptoms.[15,16]

Pouchography (luminal contrast study) can show ileoanal anastomotic separations, pouch fistulas, and anastomotic strictures. If Crohn's disease is suspected, a small bowel follow-through x-ray will rule out disease above the pouch. A computerized axial tomography (CAT) of the pelvis or magnetic resonance imaging will detect peripouch abscesses or inflammatory phlegmons. Endoluminal transpouch ultrasonography has also been used in pouch dysfunction with reported higher rates of fistula and abscess detection than both CAT scan and pouchography.[17] Anorectal manometry assesses for pelvic floor dysfunction and is another useful tool in evaluating poor pouch function. Finally, scintigraphic pelvic pouch emptying scans can be used to evaluate patients who have inefficient or inadequate pouch evacuation.[18] If a diagnosis of pouchitis is not made on endoscopic and histologic criteria and other disease states are ruled out, it is possible that the patient may have irritable pouch syndrome.[11] In these clinically symptomatic patients, treatment strategies similar to those used in irritable bowel syndrome (antidiarrheals, anticholinergics, antidepressants) may be used with some benefit.

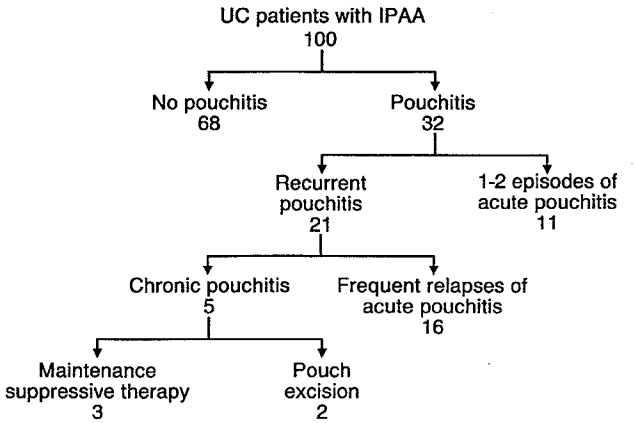

Figure 1. The clinical outcome of 100 patients at the Mayo Clinic who underwent ileal pouch anal anastomosis for ulcerative colitis. Reprinted with permission from Sandborn WJ. Pouchitis: Definition, risk factors, frequency, natural history, classification, and public health perspective. In: McLeod RS, Martin F, Sutherland LR, et al., eds. Trends in inflammatory bowel disease 1996. Lancaster, UK: Kluwer Academic, 1997:51–63.

Epidemiology, Risk Factors, and Natural History

Frequency

The cumulative risk of having one or more episodes of pouchitis varies from 15% to 53% in patients who have UC.[19–26] This wide range reflects the varied methods of defining and diagnosing pouchitis in the different studies. The rate of occurrence of a new diagnosis of pouchitis appears to be highest in the first 6 months after closure of the loop ileostomy, and then decreases significantly after 12 months.[24] The overall frequency of pouchitis is much lower in patients with FAP (3%–14%).[19,21,27] The frequency of refractory pouchitis ranges from 4.5% to 5.5%, with severe intractable pouchitis leading to excision of the pouch in 0.3% to 1.3% of patients. Figure 1 shows the clinical outcome of 100 UC patients at the Mayo Clinic who underwent IPAA.[9]

Predictive Factors

Pouchitis does not appear to have a predilection for age or race, although one small study did note a decreased incidence in African Americans when compared with Caucasians.[28] Males may have higher rates of chronic pouchitis.[29] Surgical technique does not seem to affect the frequency of pouchitis, although indication for

surgery (FAP vs. UC) does. Pouchitis rates are similar in J vs. K reservoirs,[30] S vs. W reservoirs,[31] one- vs. two-stage restorative proctocolectomy,[32] and in laparoscopic IPAA.[33]

Penna et al. found a cumulative risk of pouchitis in UC patients to be 15.5%, 22.5%, 36%, and 45.5% at 1, 2, 5, and 10 years after IPAA, respectively. This risk was much higher in patients who had primary sclerosing cholangitis (PSC), whose risk at 1, 2, 5, and 10 years was 22%, 43%, 61%, and 79%, respectively.[23] Stahlberg et al. found similar results with a cumulative risk of 51% at 4 years. All 6 patients (100%) who had PSC developed pouchitis, and extraintestinal manifestations (EIM) as a whole were a predictive factor for pouchitis.[24]

Many other studies report an increased frequency of pouchitis in patients who have EIM.[28,34–36] Seronegative arthritis responsive to steroids and associated only with active pouchitis has been reported.[37] Lohmuller et al. studied 734 patients who underwent IPAA. Patients with preoperative EIMs had a 39% incidence of pouchitis vs. 26% in those who did not. Patients who developed EIM after colectomy with IPAA had a 53% frequency of pouchitis vs. 25% in those who did not. Similar to UC, smoking may be protective against the development of pouchitis. Merrett reported a 33% frequency of pouchitis in former smokers, 25% in patients who never smoked, and 6% in current smokers.[38] These findings have been confirmed by other investigators.[24,28]

The importance of the extent of preoperative UC as a risk factor for the development of pouchitis is more controversial. Samarasekera found no relationship between distal colitis or more extensive disease and the frequency of pouchitis in 177 patients.[39] In contrast, Schmidt reported that colonic extent of disease had a significant association with the subsequent development of pouchitis after IPAA. However, the severity of the UC preoperatively was not found to be predictive.[40]

Backwash ileitis or inflammation in the terminal ileum as a risk factor for pouchitis is also controversial. One study found no correlation with development of pouchitis,[41] whereas another study found that the eosinophils and villous blunting in the terminal ileum were predictive of the degree of pouch inflammation.[40] The potential role of eosinophils is further supported by the finding of a 3-fold increase in the eosinophil concentration in preoperative colonic mucosa in patients who subsequently developed pouchitis versus those who did not.[42]

A genetic marker shown to predict the development of pouchitis is the interleukin-1 receptor antagonist gene (IL-1ra) allele 2. IL-1 is a major proinflammatory cytokine. IL-1ra competitively binds to IL-1 receptors without inducing signal transduction. However, IL-1ra allele 2 is associated with decreased levels of IL-1ra,[43] leading to an imbalance of IL-1ra/IL-1 which has been implicated in the pathogenesis of UC.[44] In a study by Carter,[45] patients who had pouchitis were found to have higher allele 2 carriage versus patients without pouchitis (72% vs. 45%). IL-1ra is not only a possible marker predicting pouchitis, but also a potential target for biologic therapy.

The predictive value of serum antineutrophil cytoplasmic antibody-perinuclear staining pattern (pANCA) is more controversial. The prevalence of pANCA in UC patients is 60%.[46] Whether this number is decreased after proctocolectomy[47,48] or unchanged[49–52] is uncertain. The literature is also divided as to whether there is a correlation between pANCA and the development of pouchitis. Four studies have found that the prevalence of pANCA is higher than expected in IPAA patients who have pouchitis (89% to 100%) and lower than expected in patients who do not have pouchitis (18% to 74%).[46,50,51,53] However, 7 more recent studies have shown that there is no correlation between pANCA and the occurrence of pouchitis.[34,48,52,54–57] Whether the failure of these later studies to show an association is based on the definitions of pouchitis used, the ANCA assay methodology, disease heterogeneity, or a true absence of association remains to be determined.

A provocative but small study by Fleshner[58] measured the quantitative levels of pANCA before colectomy for UC and divided them into high level (>100 EU/mL), moderate (40 to 100 EU/mL), and low level (<40 EU/mL). Sixty of 95 patients were pANCA-positive before colectomy, of which 9 were high-level, 32 moderate, and 19 low-level. pANCA (+) and pANCA (−) patients did not differ in the overall frequency of pouchitis (acute or chronic), and pANCA levels were not predictive of acute pouchitis. However, pANCA levels were predictive of chronic pouchitis: the cumulative risk of developing chronic pouchitis was significantly higher in patients with high-level pANCA (56%) than in moderate (22%), low-level (16%), or pANCA (−) patients (20%).[58]

PANCA levels are also increased in patients who have PSC.[59] PSC, in turn, is a risk factor for pouchitis.[23,47,60] Patients who have PSC and who undergo IPAA have a 63% chance of developing pouchitis versus only 32% for those who do not have PSC. The cumulative risk of developing pouchitis in patients who have PSC is also higher at 1, 2, 5, and 10 years than that in patients who have UC and do not have PSC.[23] The increased incidence of pouchitis in patients who have PSC and other EIM suggests that there may be a particular genotype of UC

Table 3. Predictive Factors for the Development of Pouchitis

1. Male gender (chronic pouchitis)
2. Primary sclerosing cholangitis
3. Extraintestinal manifestations
4. Nonsmoker
5. Extent of colitis[a]
6. Backwash ileitis[a]
7. Preoperative quantitative pANCA level (chronic pouchitis)[a]
8. IL-1ra gene allele 2

[a]Denotes that the data is mixed.

that has a stronger predisposition to develop pouchitis. PANCA may or may not be a serological marker for that genotype. These correlations also support the theory that pouchitis may be either a recurrence of UC in the pouch or a third, new form of inflammable bowel disease (IBD). Table 3 summarizes the potential predictive factors for the development of pouchitis.

Quality of Life

Aside from pouchitis, outcome after IPAA is variable and is dependent on surgical expertise. Most studies report an average of six bowel movements a day and some fecal incontinence in approximately 50% of patients.[61,62] Despite these numbers, the health-related quality of life (QOL) after IPAA has consistently been comparable to normal populations and is better than in active UC.[63–66] However, poor functional status, increased number of bowel movements, and chronic pouchitis do decrease health-related QOL.[66] Improved QOL overall after surgery but a worse QOL with pouchitis[67] has been confirmed by use of the Cleveland Global Quality of Life score, a tool specifically developed to assess patients with a restorative proctocolectomy.[68] The IBD questionnaire,[69] a QOL tool validated in UC and Crohn's disease, appears to correlate with PDAI and is another tool that can be used to measure QOL in patients who have pouchitis.[70]

Complications

The effect of acute pouchitis on long-term functional results is not clear. Whereas one prospective study of 137 patients found that even one episode of acute pouchitis can result in poorer long-term functional results,[20] Keranen et al. found that only chronic pouchitis affects functional outcomes.[22] Chronic pouchitis is rarely a cause for pouch excision.[62,71] Women who have IPAA have significantly lower fertility rates than those who have UC,[72] and while pregnant have poorer QOL scores[67] with transient worsening of pouch function.[73] The contribution of pouchitis to this is unknown.

Metabolic sequelae after IPAA have been found to be associated with pouchitis and include decreased levels of albumin, calcium, total cholesterol, triglycerides, and vitamin E. Vitamin A, B_{12}, and D deficiency have also been found.[74] Osteopenia has been found using bone densitometry testing in patients who have villous atrophy of the ileal reservoir, a hallmark of pouchitis.[75]

Etiology

The etiology of pouchitis is unknown. Speculation has centered on the role of genetic susceptibility, fecal stasis, and/or bacterial overgrowth, an altered balance of luminal bacteria (dysbiosis), nutritional deficiencies, ischemic complications of surgery, a novel third form of IBD, a recurrence of UC in the pouch, or a missed diagnosis of Crohn's disease. The significantly higher occurrence of pouchitis in patients who have UC versus FAP suggests that the mechanism is not related to surgical changes common to both diseases (i.e., ischemia and fecal stasis). However, the efficacy of antibiotics and probiotics in treating pouchitis suggests that the latter mechanism may play a role. The ileal pouch undergoes adaptive changes once it is exposed to the fecal stream. Functionally, it changes from a primarily absorptive organ to an organ of storage. The histopathologic changes that follow reflect this transition. Ileal pouches acquire certain colonic characteristics such as goblet cells, villous atrophy, and crypt hyperplasia; however, complete colonic metaplasia does not seem to occur.[76,77] The UC host may be genetically more susceptible to having an inflammatory response to insults in their adapted pouch mucosa, much as they are thought to be susceptible to such insults in their now resected colon. Table 4 summarizes the potential etiologies of pouchitis.

Treatment

The treatment of pouchitis is predominantly empiric given the few controlled trials available. To date, there have been at least 9 published controlled trials on the treatment of pouchitis.[78–86] Antibiotics are the mainstay of acute and chronic treatment, but probiotics may play a role in the maintenance of remission in chronic pouchitis. Table 5 lists the treatment options currently available.

Antibiotics

Metronidazole and ciprofloxacin are the first-line therapy for pouchitis. Evidence that metronidazole is effective comes from an "N-of-1" randomized trial[78] and a randomized controlled crossover trial which showed a 73% response (defined as a decrease in stool frequency) in 13 patients with chronic pouchitis. The placebo response was 9%.[79] Hurst et al. found that 41 of 52 patients

Table 4. Potential Etiologies in the Development of Pouchitis

Cause	Supportive evidence	Negative evidence	Reference nos.
Altered immunoregulation	+/− pANCA IL-1ra gene allele 2 ↑ Lymphocyte densities ↑ Inflammatory cytokines Extraintestinal manifestations		45, 58, 135, 136
Crohn's disease	Ileal inflammation Fistulas	Disease in pouch only	92
Fecal stasis Bacterial overgrowth Dysbiosis	Antibiotics Probiotics	Same bacterial count w/ or w/o pouchitis	137–140
Fecal bile acids		Same total bile acid concentration in pouchitis vs. healthy	92, 140, 141
Short chain fatty acids		No correlation between SCFA, pouchitis, fecal bacterial concentrations	140
Ischemia	↓ Mucosal blood flow	Same surgery as FAP Allopurinol ineffective	83, 142

(79%) with acute pouchitis responded to a 7-day course of metronidazole at 250 mg orally 3 times a day with complete relief.[27] Two small series found metronidazole to have a response rate of 100% when given as a topical solution instilled at 75 to 150 mg daily[87] or 40 to 160 mg daily.[88]

Hurst reported that 11 of 52 patients did not respond to metronidazole. These patients were then given ciprofloxacin 500 mg twice a day, of whom 8 (73%) responded. Thus, the overall antibiotic response rate was 96%.[20] A randomized trial by Shen[85] compared 2 weeks of treatment with metronidazole 20 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} to ciprofloxacin 1000 mg/day in patients who had acute pouchitis. Both drugs significantly reduced the PDAI score, but ciprofloxacin had a greater reduction in overall PDAI score (6.9 $\pm$ 1.2 vs. 3.8 $\pm$ 1.7, $P = .002$), symptom score (2.4 $\pm$ 0.9 vs. 1.3 $\pm$ 0.9, $P = .03$), and endoscopic score (3.6 $\pm$ 1.3 vs. 1.9 $\pm$ 1.5, $P = .03$) vs. metronidazole. None of the patients who were administered ciprofloxacin experienced side effects whereas 33% of the patients who were administered metronidazole had adverse events. The side effect profile of metronidazole includes dysgeusia, dyspepsia, nausea, and peripheral neuropathy. For many practitioners, these undesirable sequelae of therapy have made ciprofloxacin the drug of choice for pouchitis therapy. Other antibiotics used with anecdotal success include amoxicillin/clavulanic acid, erythromycin, and tetracycline.[89]

In patients who have chronic recurrent or refractory pouchitis, antibiotic combination therapy may be effective. Gionchetti used rifaximin 1 g twice daily in combination with ciprofloxacin 500 mg twice daily for 15 days in 18 patients who had chronic treatment resistant pouchitis.[90] Six of 18 (33%) had complete remission defined as a PDAI of 0. Ten of 18 (55.6%) had clinical improvement with a decrease of 3 points on their PDAI score, for a total response rate of 88.8%. An open-label trial of metronidazole 400 to 500 mg twice daily, plus ciprofloxacin 500 mg twice daily for 28 days in patients who had recurrent or treatment refractory pouchitis noted an 82% remission rate. The median PDAI scores before and after therapy were 12 (range, 8 to 17 points) and 3 (range, 1 to 10 points), respectively.[70]

An initial episode of pouchitis should be treated with ciprofloxacin 500 mg twice daily or metronidazole 250 mg 3 times a day for 7 to 10 days. Response should be seen within 2 to 3 days. Responding patients who experience recurrent episodes and are able to tolerate the medication should be retreated with the same regimen. Some patients who have chronic pouchitis will require anywhere from 500 mg of ciprofloxacin or 250 mg of metronidazole every third day to 500 mg ciprofloxacin twice daily or 250 mg metronidazole 3 times daily to maintain their response. Others may develop resistance and require combination antibiotic therapy or a rotating schedule of 3 or more antibiotics. If antibiotics fail, other therapeutic options should be considered. Patients who have chronic pouchitis should be considered for probiotic therapy as described below (Figure 2).[91]

Mesalamine

Anecdotal reports suggest a benefit from topical mesalamine.[12,92,93] Miglioli et al. describe three patients who had pouchitis after IPAA for UC. They were administered mesalamine as a suppository or enema at 1.2

Table 5. Treatment Options

Class	Efficacy	Example
1. Antibiotics	+ Acute pouchitis + Chronic pouchitis	A. Metronidazole[a] B. Ciprofloxacin[a] C. Amoxicillin/clavulanic acid D. Erythromycin E. Tetracycline F. Rifaximin + ciprofloxacin G. Metronidazole + ciprofloxacin[a]
2. Probiotics	+ Prophylaxis + Maintenance	A. VSL #3[a] B. *E. coli* Nissle 1917
3. Mesalamine	+/−	A. Mesalamine enemas B. Sulfasalazine C. Oral mesalamine agents
4. Corticosteroids	+/−	A. Corticosteroid enemas B. Budesonide suppositories C. Budesonide enemas[a] D. Oral corticosteroids
5. Nutritional agents	+/−	A. SCFA enemas/ suppositories[b] B. Glutamine suppositories[b] C. Inulin[a]
6. Immune modifier agents/ biologics	+/−	A. Cyclosporine enemas B. Azathioprine/6-mercaptopurine C. Infliximab
7. Oxygen free radical inhibitor	− prophylaxis	A. Allopurinol[b]
8. Smoking/ nicotine	+	A. Smoking B. Transdermal nicotine (?)
9. Antidiarrheal/ antimicrobial	+/−	A. Bismuth subsalicylate B. Bismuth carbomer enemas[b]
10. Surgical options		A. Ileal pouch exclusion B. Ileal pouch excision

[a]Denotes positive randomized controlled trial.
[b]Denotes negative randomized controlled trial.

to 4 g daily. After 20 to 30 days, clinical and endoscopic improvement was noted with partial histological recovery.[94]

The bacteria required to split the azo-bond in sulfasalazine and release the mesalamine moiety is present in the reservoir of patients after IPAA,[95] suggesting that sulfasalazine is a rational treatment modality. Pentasa may also achieve some release of mesalamine into the ileal pouch. However, there are no randomized controlled trials of any oral mesalamine agents for the treatment of pouchitis.

Corticosteroids

When antibiotics fail, oral and topical corticosteroids have been tried with limited anecdotal success.[92,93] A small open trial of budesonide suppositories was conducted in 10 patients who had active pouchitis. After budesonide 1.5 mg per day for 4 weeks, all patients had clinical and endoscopic improvement or remission, but 6 (60%) relapsed within 8 weeks.[96] A randomized, placebo-controlled trial of 2-mg budesonide enemas versus metronidazole also showed efficacy.[84] Twenty-six patients who had acute pouchitis by PDAI score ≥ 7 were randomized to either budesonide enemas or oral metronidazole 500 mg twice daily for 6 weeks. Fifty-eight percent of budesonide patients and 50% of metronidazole patients improved. Fifty-seven percent of metronidazole patients had adverse events versus only 25% of budesonide patients. Oral-controlled release budesonide has not been reported for the treatment of pouchitis, but anecdotal experience suggests that it may be effective (W. J. Sandborn, unpublished data, December 2002).

Immunosuppressive Therapy

MacMillan reported a small retrospective series of 4 patients who had chronic pouchitis that were treated with azathioprine or 6-mercaptopurine.[97] Patients were able to discontinue steroids and maintain a sustained response for up to 3 years. Immunosuppressive therapy is not protective against the development of pouchitis in the posttransplant setting. Zins reported 7 patients who had IPAA who underwent orthotopic liver transplanta-

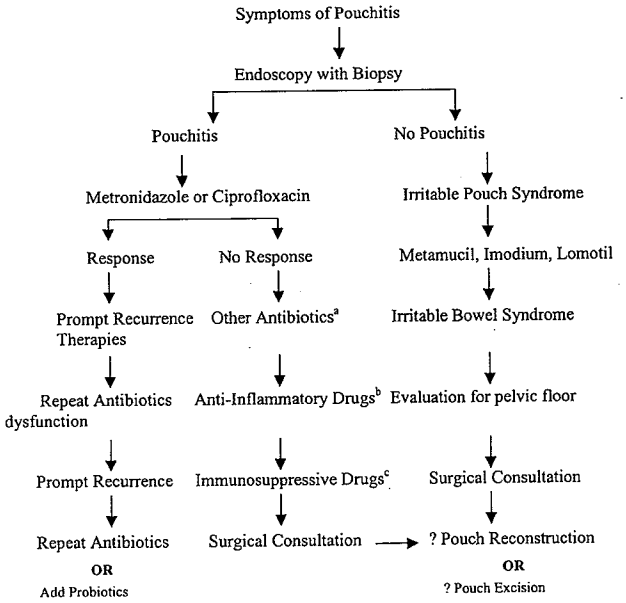

Figure 2. Treatment algorithm for pouchitis. [a]Other antibiotics indicates: rifaximin; amoxicillin/clavulanate; erythromycin; tetracycline; and cycling of multiple antibiotics. [b]Anti-inflammatory drugs indicates: bismuth subsalicylate, mesalamine enemas, sulfasalazine, and oral mesalamine. [c]Immunosuppressive drugs indicates: budesonide, steroid enemas, oral steroids, azathioprine.

tion for PSC.[98] Five of 7 had chronic or recurrent pouchitis before transplant, of whom 4 continued to have chronic pouchitis after transplant despite a triple immunosuppressive regimen of prednisone, azathioprine, and either cyclosporine or FK 506. One patient who had been free of pouchitis before transplant developed a single acute episode posttransplant. Similarly, Rowley reported that 1 of 4 patients with an orthotopic liver transplant for PSC who underwent colectomy with IPAA for UC developed chronic pouchitis despite immunosuppression with cyclosporine.[99]

Infliximab has been reported to be of benefit for treating Crohn's disease in the ileal pouch.[100] More recently, Arnott[101] reported that 2 patients who had refractory pouchitis responded to a single infusion of infliximab (response defined as a decrease in the number of bowel movements and less urgency) with benefit sustained to 12 weeks. No long-term follow-up information was provided.

Bismuth

Bismuth-containing carbomer foam enemas showed promising results in an open label trial.[102] Twelve patients who had treatment refractory chronic pouchitis were treated with 230 mg elemental bismuth-containing carbomer foam enemas. The enemas were given nightly for 45 days. Ten of 12 (83%) patients had a clinical response with a decrease in their PDAI scores by 2 points or more. Of these 10, 6 (60%) maintained their response over 12 months while receiving an enema every third night. No side effects were reported. Unfortunately, a randomized double-blind placebo control trial in 40 patients did not show a difference between placebo and bismuth carbomer foam enema in the treatment of chronic pouchitis.[81] Twenty patients received a placebo enema containing a gum resin and 20 patients received 270 mg of elemental bismuth complexed with carbomer delivered as foam enemas for 3 weeks. No patients achieved remission (PDAI of 0) but 9 patients (45%) in each group achieved a clinical response with a 3-point decrease in their PDAI. The investigators cite low concentrations of bismuth in the enemas, short duration of treatment, therapeutic efficacy of gum resin (given the high placebo rate of 45%), or a true treatment failure to explain the lack of efficacy of bismuth.

A retrospective series of 13 patients who had chronic pouchitis studied the effect of oral bismuth subsalicylate tablets (Pepto-Bismol, Proctor and Gamble, Cinncinati, OH) on disease course. All patients were receiving antibiotics (metronidazole or ciprofloxacin) but remained symptomatic. All patients received an initial dose of eight 262-mg chewable bismuth subsalicylate tablets per day for 4 weeks. Eleven of 13 had a clinical response with a decrease in stool frequency, fecal incontinence, and/or abdominal cramping. One patient reduced their dose secondary to bloating, while the 7 others reduced their dose because of similar benefit at the lower dose. Five of 11 responders were able to discontinue antibiotic use after 4 weeks.[103] These inconsistent results with bismuth indicate that an additional controlled trial of oral bismuth may be warranted.

Allopurinol

Allopurinol is a xanthine oxidase inhibitor. The theoretical basis for its use in pouchitis is to inhibit the production of free radicals and thus inhibit mucosal injury. A small trial by Levin et al. showed a 50% response rate in acute and chronic pouchitis.[104] Eight patients who had acute pouchitis received 300 mg twice daily of allopurinol. Four had resolution of symptoms. Fourteen patients who had chronic pouchitis were treated with the same dose for 28 days; 7 of 14 had a clinical response. However, a randomized controlled trial of allopurinol for the prophylaxis of pouchitis was negative.[83] In this study, 184 patients who had UC who were undergoing IPAA were randomized to receive postoperative allopurinol 100 mg twice daily or placebo. The cumulative risk of pouchitis was 31% in the allopurinol group and 28% in the placebo group, which was not significant. Additionally, there was no difference in overall pouch function between these 2 groups. These findings do not lend credence to the theory of ischemic damage and free radical injury contributing to the pathogenesis of pouchitis.

Nutritional Agents

Fiber. Thirlby et al. showed that oral fiber supplementation with either pectin, a soluble fermentable fiber supplement, or Citrucel (Glaxo Smith Kline, Research Triangle Park, NC), a methyl cellulose-based, nonfermentable fiber, has no benefit on stool frequency, pouch function, bloating, and stool consistency in patients after IPAA.[105] Inulin, a dietary fiber that is fermented to short-chain fatty acids (SCFA), was studied in a randomized placebo-controlled trial of 3 weeks duration.[86] Pouch patients receiving 24 g/day of inulin had increased butyrate concentrations (18.9 vs. 11.7, $P = 0.01$), decreased fecal pH (5.33 vs. 5.62, $P = 0.02$), decreased concentrations of *Bacteroides fragilis* (6.77 vs. 7.68, $P = 0.02$), and lower levels of some secondary bile acids in the feces compared with patients on placebo. The overall PDAI score was lower in inulin-treated patients (4.05 vs. 5.39, $P = 0.01$) than in placebo, with significantly lower endoscopic (0.95 vs. 1.47, $P = 0.04$) and

histologic scores (2.11 vs. 2.61, $P = 0.04$), but no difference in the clinical score (1.00 vs. 1.26, $P = 0.17$). However, because all of these patients did not meet the definition of pouchitis by PDAI score and there was no significant improvement in clinical symptom scores, the actual benefit to the patient of receiving inulin therapy is unclear.

Short chain fatty acids/glutamine. SCFA (acetate, propionate, butyrate) are produced by anaerobic bacterial fermentation. They are the major source of energy for the colonic mucosa.[106] Glutamine is the analogous energy source for the small intestinal mucosa. Studies reporting the use of SCFA as a treatment for pouchitis are limited, and the results are mostly negative. Two small series used the same SCFA enema formulation of 60 mmol/L sodium acetate, 30 mmol/L sodium propionate, and 40 mmol/L sodium *n*-butyrate in a combined total of 10 patients who had chronic pouchitis.[107,108] Only 3 patients had a clinical response whereas 2 patients actually had worsening of their clinical symptoms. Den Hoed described a single patient who had refractory pouchitis who completely responded to treatment with a similar SCFA enema.[109] Another study randomized patients with chronic pouchitis to either butyrate or glutamine suppositories for 10 days. Three of nine (33%) patients whose symptoms were treated with butyrate and 6 of 10 (60%) patients whose symptoms were treated with glutamine responded.[82] Given the lack of a placebo control, it is unclear whether these two therapies are similarly effective or similarly ineffective.

Smoking/Nicotine

Current smoking has been reported to be protective against pouchitis.[24,28,38] To date, there have been no trials of nicotine enemas or transdermal nicotine patch for the treatment of pouchitis.

Probiotics

Probiotics are live organisms, typically bacteria, found as commensals in the human gastrointestinal tract. Based on the hypothesis that an imbalance in the usual fecal flora (dysbiosis) may result in inflammatory conditions such as pouchitis, Gionchetti conducted a randomized double-blind placebo controlled trial of the probiotic formulation VSL-3 (Sitia-Yomo, Milano, Italy).[110] Forty patients who had chronic pouchitis in remission after treatment with antibiotics (PDAI = 0) received either placebo or a 6 g daily oral dose of VSL-3 for 9 months. VSL-3 contains 5×10^{11}/g of viable lyophilized bacteria consisting of 4 strains of lactobacilli (*L. acidophilus, L. delbrueckii* subsp. *bulgaricus, L. plantarum, L. casei*), three strains of bifidobacteria (*B. infantis, B. longum, B. breve*) and one strain of *Streptococcus salivarius* subsp. *thermophilus*. Seventeen of 20 patients (85%) who were treated with VSL-3 maintained remission (relapse was defined as an increase in the PDAI $\geq$ 2 points) compared to none of 20 patients who were treated with placebo. No adverse events were reported. The VSL-3–treated group was found to have an increase in fecal concentrations of lactobacilli, bifidobacteria, and *S. thermophilus* by day 15. A second controlled trial of VSL-3 for the treatment of chronic pouchitis was conducted in 36 patients with similar results.[111] VSL-3 is also more effective than placebo as a prophylaxis against the development of pouchitis in the first year after surgery.[112] A case report of 2 patients suggested that another probiotic, *Escherichia coli* strain Nissle 1917, may be of benefit for the treatment of active pouchitis and the maintenance of remission as well.[113]

The mechanism of action of probiotics in pouchitis is unclear. Patients who have pouchitis and who received probiotic therapy with VSL-3 were found to have increased concentrations of the anti-inflammatory cytokine IL-10 and a reduction of the proinflammatory cytokines IL-1α, interferon-γ, and tumor necrosis factor-α, as well as inducible nitric oxide synthase and matrix metalloproteinase activity to concentrations similar to those found in noninflamed pouches.[114] *E. coli* Nissle 1917 was able to induce IL-8 while VSL-3 was not, suggesting that these 2 probiotic formulations may have different modes of action.[115]

Crohn's Disease

When Crohn's disease is diagnosed in the pouch (based on pre-pouch ileitis or fistula involving the pouch), treatment is similar to the treatment of Crohn's disease elsewhere in the gastrointestinal tract. Berrebi reported on 2 patients who had IPAA and were diagnosed with Crohn's disease in the reservoir. Both responded to corticosteroid and azathioprine therapy, with eventual maintenance on azathioprine alone.[116] Ricart reported a series of 7 patients who had IPAA for UC who were subsequently diagnosed with Crohn's disease and who were refractory to conventional therapy. These patients were treated with infliximab. Six patients had a complete response with closure of all fistulous tracts, and one had a partial response.[100]

Pouch Excision

Pouch excision is rare and occurs more commonly for pouch dysfunction than for true chronic pouchitis. However, Penna et al. estimate that approximately 1.3% of patients who undergo IPAA for UC will need a pouch excision for chronic treatment refractory pouchitis.[23]

Dysplasia

There have been at least 17 cases of adenocarcinoma arising in the permanent (Brooke) ileostomy of patients who had UC. The case described by Reissman notes diffuse colonic metaplasia in the ileostomy around the adenocarcinoma with sulfomucin production.[117] In 1997, the first case of an adenocarcinoma arising in a continent ileostomy (Kock pouch) was described in a patient who had UC. The pouch mucosa showed chronic inflammation with villous atrophy and mild to moderate dysplasia.[118] It was not clear if this patient suffered from recurrent pouchitis. Also in 1997, a case of large cell lymphoma arising in the pouch of a patient who had had UC was described. This patient suffered from chronic refractory pouchitis, which may in retrospect have been due to the invasive lymphoma, undetected until surgical resection of the pouch for pouch dysfunction.[119]

Rectal cancer has developed after IPAA in the residual columnar epithelium or rectal cuff.[120–122] Although this makes intuitive sense, the risk of dysplasia and adenocarcinoma developing in the ileal reservoir has been mostly a theoretical concern. However, dysplasia has now been noted by 3 groups in the ileal reservoir including the development of adenocarcinoma of the pouch in one patient who had chronic pouchitis.[123–126]

In 1991, Lofberg et al.[127] reported the first case of pelvic pouch dysplasia. The patient was a 36-year-old man who underwent a colectomy, mucosal proctectomy, and IPAA with a S-type pelvic pouch. No dysplasia was noted in the colectomy specimen. The patient suffered from chronic pouchitis and was on long-term metronidazole therapy. Four years after pouch creation, he was noted to have low-grade dysplasia on biopsy and DNA aneuploidy by flow cytometry. The patient then underwent periodic surveillance pouchoscopy with biopsy, and in 1996 high-grade dysplasia was detected.[126] In 1997, the patient was diagnosed with primary cholangiocarcinoma, with likely underlying subclinical PSC.[128]

In 1995, the same group reported the results of 87 patients who had IPAA for UC whose cases were followed for a mean of 6.3 years. Three types of mucosal adaptation were noted in the reservoir. Type A (51% of patients) was characterized by normal mucosa or a mild villous atrophy and no or mild inflammation. Type B (40% of patients) showed transient atrophy with temporary moderate or severe villous atrophy followed by normalization. Finally, Type C (9%) showed constant atrophy with permanent total or subtotal villous atrophy accompanied by severe pouchitis. It was in this last group that low-grade dysplasia was found in 3 of 8 patients. This group also had the highest level of sulfomucin-producing goblet cells in the pouch.[124] A prospective follow-up study of 7 patients who had Type C mucosa and 14 who had Type A patterns was performed. Dysplasia was noted in 5 of 7 Type C pouches (71%) (4 low-grade dysplasia and 1 high-grade dysplasia). There was no correlation with dysplasia in the colectomy specimen, but there was an association with an early onset of UC. The investigators believed that patients who were identified as having a Type C response 4 years after ileostomy closure should have at least annual pouchoscopy with surveillance for dysplasia.[126]

Other investigators have not found dysplasia on surveillance of the pouch,[129–132] but have found similar rates of Type A, B, and C mucosa in adults[131] and children[132] who have an IPAA for UC. Setti Carraro confirmed the finding that only patients who had Type C mucosa developed chronic pouchitis. He also noted that the categorization of response type could be made at 6 months after ileostomy closure.[131] In a study of six patients who had chronic severe pouchitis, one had a genetic alteration associated with colorectal carcinoma, a loss of heterozygosity at 5q15-22.[133]

In 2001, Thompson-Fawcett[123] surveyed the pelvic pouches of 106 patients who had potential risk factors for dysplasia—chronic pouchitis, pelvic pouch for 12 years or more, Kock pouch for 14 years or more, and neoplasia in the colectomy specimen. One patient who had a long-standing pouch had multifocal low-grade dysplasia. She had never had an episode of pouchitis and opted for pouch excision.

In 2000, Iwama reported a case of adenocarcinoma in a J-pouch that had been outside of the fecal stream for 18 years.[134] In 2001, Heuschen et al. reported the first adenocarcinoma of a functioning pelvic pouch that clearly developed from the ileal mucosa.[125] This was a patient who had pancolitis and backwash ileitis who underwent IPAA for multifocal dysplasia. The patient developed chronic pouchitis and was noted to have a tubulovillous neoplasia on pouch biopsy 3 years after surgery. Pouch excision was performed and a flat carcinoma in the proximal pouch was found.

Overall, pouch dysplasia is very rare. No screening program is currently advocated for patients with IPAA after colectomy for UC. Further studies are needed to delineate which patients need screening, when, where within the pouch, and how often. Potential risk factors for pouch dysplasia may be dysplasia in the original colectomy specimen, chronic pouchitis, and the age of the pouch. It is reasonable, based on the available data, to perform random mucosal sampling in the reservoir of all

patients who have a history of UC and a pouch 1 year after closure of the ileostomy. Those found to have Type C mucosal changes and/or chronic pouchitis should undergo annual surveillance pouchoscopy, as is done for patients who have UC. Patients who have dysplasia on colectomy may need to be surveyed regardless of evidence for chronic pouchitis.

Summary

Pouchitis is an idiopathic inflammatory disease of the ileal reservoir in patients who have undergone IPAA. Approximately half of all UC patients who undergo this procedure will have at least 1 episode of pouchitis with approximately 15% experiencing a chronic course. PSC and other EIM increase the likelihood of developing pouchitis, whereas smoking is protective. Similar genetic and autoimmune mechanisms to UC appear to occur in an ileal reservoir that shows increasingly colon-like adaptations with respect to bacterial content and mucosal characteristics. Although most patients have a good response to antibiotic therapy, increasing evidence supports a role for probiotics in prevention and maintenance. Finally, dysplasia is a rare but real concern, and pouch surveillance guidelines must be developed.

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Exhibit J

Clinical review

Regular review

Ulcerative colitis

Subrata Ghosh, Alan Shand, Anne Ferguson

Ulcerative colitis is a relapsing and remitting disease characterised by acute non-infectious inflammation of the colorectal mucosa. In the United Kingdom the annual incidence is around 7 cases per 100 000 population.[1] The rectal mucosa is invariably affected. Confluent inflammation and shallow ulceration extend proximally from the anal margin. A patient may have proctitis, left sided colitis (the proximal limit being below the splenic flexure), extensive colitis (involving the transverse colon), or pan colitis. At any point in time, 50% of patients are asymptomatic, 30% have mild symptoms, and 20% have moderate to severe symptoms.[2] Many patients have long periods of complete remission, but the cumulative probability of remaining free from relapse at two years is only 20%, decreasing to less than 5% at 10 years.[3] Later relapses generally affect the same region of the colon as previous episodes.

Several of the current clinical and therapeutic issues in ulcerative colitis include: (*a*) medical treatment options for relapse and for maintenance of remission; (*b*) management of the minority of patients who develop a severe life threatening relapse or chronic unremitting disease; (*c*) surgical treatment of ulcerative colitis; and (*d*) long term complications in patients with extensive disease—namely, colonic and biliary cancers and sclerosing cholangitis.

Summary points

Ulcerative colitis may present at any age, but the anatomical distribution of involvement at presentation is different between children and adults

All patients with bloody diarrhoea need to have infection excluded

Outpatient rigid sigmoidoscopy is the best method of diagnosing the nature of inflammation

The extent of inflammation may be established by total colonoscopy (or a double contrast barium enema)

The mainstays of treatment are rectal and systemic 5-aminosalicylic acid derivatives and corticosteroids, with azathioprine in steroid dependent or resistant cases

Restorative proctocolectomy with ileal pouch-anal anastomosis should be considered in every patient in whom colectomy is contemplated

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Methods

We have based this review on our clinical and research experience in gut immunology and inflammatory diseases together with information from comprehensive monographs[3–5] and UK and US guidelines on management.[6,7] We also searched Medline from 1985 to July 1999 using the search terms "ulcerative colitis" and "sclerosing cholangitis," which yielded 6116 and 889 citations respectively and other seminal papers.

Clinical features and diagnosis

Ulcerative colitis may present at any age. Men and women are equally affected. In adults at presentation about 55% have proctitis, 30% left sided colitis, and 15% extensive colitis or pan colitis. In children, only 25% present with proctitis alone, 30% have left sided colitis, and in 45% the disease extends to the transverse colon or beyond.

Box 1 lists typical symptoms at presentation. Virtually all patients with ulcerative colitis have rectal bleeding or bloody diarrhoea. Delays in presentation are common through such diverse reasons as fear of cancer or a general reluctance to discuss matters relating to bowel habit (box 2). Many patients may complain of pain of colonic origin, often left sided and related to defecation. There are no specific clinical signs on general examination, but inflammation of the rectal mucosa (proctitis) can readily be seen at proctoscopy (fig 1).

Patients with bloody diarrhoea need careful clinical evaluation. After infection has been excluded in patients with colitis the nature and extent of inflammation should be established by sigmoidoscopy and biopsy and either total colonoscopy or double contrast barium enema examination (fig 2). Sedation may be necessary for appraisal of the rectal mucosa in an anxious child presenting for the first time. The patient should be informed that rectal examination and sigmoidoscopy are safe and usually painless and that they are performed routinely at appointments to allow sensible treatment decisions to be made. Sigmoidoscopy may be

Box 1: Symptoms of ulcerative colitis

Colonic symptoms
- Diarrhoea with blood and mucus
- Urgency, tenesmus
- Incontinence
- Lower abdominal cramps and pain with defecation

Systemic symptoms
- Tiredness, weight loss
- Malaise, fever

Extraintestinal

Related to activity of colitis
- Peripheral arthritis
- Erythema nodosum
- Iritis, uveitis
- Thromboembolism

Unrelated to activity of colitis
- Sclerosing cholangitis
- Ankylosing spondylitis, sacroileitis
- Pyoderma gangrenosum (see fig A on website)

performed safely during pregnancy if considered essential for management, but never total colonoscopy.

Differential diagnosis

The most difficult decision may be to establish whether the diagnosis is ulcerative colitis or Crohn's disease. It may be several years after presentation that the clinical evolution allows a firm decision to be made. Fortunately, unless surgery is contemplated the management of colonic Crohn's disease is broadly similar to that of ulcerative colitis. The table summarises the differences between ulcerative colitis and Crohn's disease. In our view these are different diseases. Otherwise the differential diagnosis includes anal fissure (seen with proctoscopy), infectious colitis (stool cultures for bacterial pathogens, and careful examination of stools and biopsy material for viral, parasitic, and protozoal infection are mandatory), and pseudomembranous colitis (history of antibiotic exposure, toxin assay for *Clostridium difficile*). Food sensitive colitis should be considered in infants[8] and ischaemic colitis, diverticulitis, and colonic tumours in adults.

Causal and immunological aspects

The cause remains unresolved, but current interest is focused on defects in the mucous gel barrier, either primary or acquired by bacterial sulphatases,[9-11] low appendicectomy rates in ulcerative colitis[12] (even when smoking is controlled for),[13] and colonic sulphate reducing bacteria.[14] The existence of true autoimmunity in ulcerative colitis is uncertain, and the evidence is conflicting.[15,16] The balance between Th1 and Th2 phenotypes of T lymphocytes determines the characteristics of a chronic inflammatory process. Th1 cells secrete proinflammatory cytokines such as interleukin 2 and γ interferon, whereas Th2 cells express regulatory cytokines such as interleukin 4 and interleukin 10. Th2 responses have been shown to be important in atopy, a condition in which altered humoral immunity is present, and existing data suggest that ulcerative colitis more closely resembles a Th2 type disease.[17]

Box 2: Atypical symptoms at presentation or relapse
- Bleeding not recognised because of colour blindness
- Constipation proximal to severe proctitis—blood and mucus several times daily, hard stool once or twice a week (olsalazine frequently causes diarrhoea and may be a useful drug)
- Late stage tubular colon with failure of the capacity to concentrate stool so the patient has watery diarrhoea without inflammation or bleeding
- Faecal incontinence—at least 50% of patients with diarrhoea due to ulcerative colitis are occasionally incontinent
- The patient does not recall an earlier episode of colitis but presents with sclerosing cholangitis or colon cancer and dysplasia (even if there are no current symptoms of ulcerative colitis, biopsies usually show evidence of a previous total colitis)
- Chronic iron deficiency anaemia—usually there is a history of diarrhoea on direct questioning

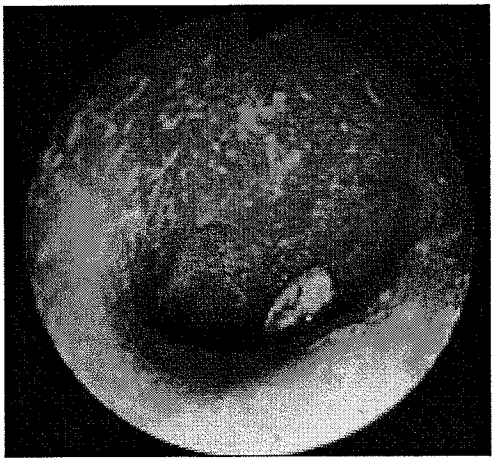

Fig 1 Appearance of rectum in proctitis with erythematous friable mucosa, loss of vascular pattern, and mucopus

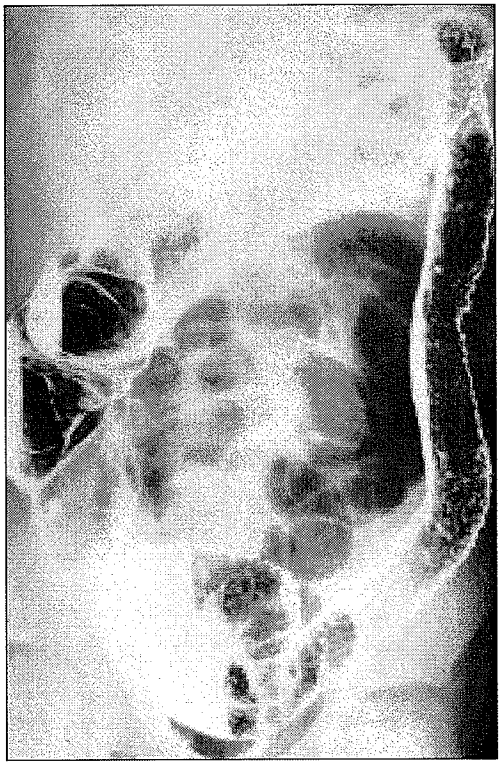

Fig 2 Double contrast barium enema showing granularity and continuous involvement with ulcerations

Management

Analysis of the patient's illness

As in many chronic diseases, an appropriate plan of management must be tailored to the patient's current anatomical, functional, and "disease activity" status. Anatomical extent of grossly affected colon, symptomatic disease activity, local and remote complications, iatrogenic illnesses, nutrition, growth variables, social and psychological factors, and coexistent diseases should all be considered within a comprehensive management strategy.

Symptoms are the best guide towards disease activity[18] and their relief is the main treatment aim. It may be difficult to decide whether remission has been achieved, and this is a major problem in the design of clinical trials. Various clinical indices have been devised, mainly based on subjective data. The Powell-Tuck index[19] is widely employed and is of particular use in clinical trials where objective, reproducible assessment of symptoms is vital. In practice, the Crohn's disease activity index[20] gives similar values to the Powell-Tuck index (see fig B on website). Symptoms may remit but endoscopically there may still be evidence of mucosal inflammation; histology often remains abnormal long after complete clinical remission. Blood tests indicating inflammatory activity, such as platelet and leucocyte counts, erythrocyte sedimentation rate, or concentrations of C reactive protein, although a useful adjunct, often merely confirm the overall clinical impression. A new objective measure of gut inflammation (gut protein loss measured by lavage fluid from the whole gut) measures the same symptomatic, acute inflammatory component of overall illness as the Crohn's disease activity index, both in ulcerative colitis and Crohn's disease in adults (see fig C on website).[21] Tests based on whole gut lavage offer a different approach for assessing the contribution of "disease activity" to overall illness, as well as measuring the efficacy of treatment.

Medical treatment of typical relapse

In practice, rectal and systemic derivatives of 5-aminosalicylic acid and corticosteroids form the basis of medical treatment (see table on website). Azathioprine may be used as a steroid sparing agent. There is little to choose between the various 5-aminosalicylic acid preparations available. The use of sulphasalazine, the oldest (and least expensive) of these, has become less popular because of side effects including nausea, skin rashes, and reversible oligospermia. Balsalazide, a colonic release preparation that is azo bonded may be more effective and better tolerated than mesalazine.[22] Topical treatment is usually effective for proctitis. Patients may need to be taught how to use formulations given through the rectum. Rectal 5-aminosalicylic acid preparations and corticosteroids are both effective in relieving symptoms and inducing remission but the former is more effective.[23] Patients tolerate foam enema preparations better than liquid enemas,[24] but the new mesalazine gel enema may be better still.[25]

There is no evidence that elemental diets or other dietary intervention have any specific therapeutic effect in ulcerative colitis. However, the support of a dietician in the management of patients is invaluable for monitoring daily nutritional intake and educating patients on the principles of good nutrition. Many patients are iron deficient and may require supplementation with oral iron preparations. Parenteral iron or recombinant human erythropoietin[26] has been used in cases where oral supplements are poorly tolerated.

Differences between ulcerative colitis and Crohn's disease

	Ulcerative colitis	Crohn's disease
Disease features		
Smoking	Non-smokers or ex-smokers	Smokers
Long latent period	No	Yes
Genetic susceptibility	+; HLA class II	++; non-major histocompatibility complex
Osteopenia at diagnosis	No	Yes
Oral and perianal disease	No	Yes
Growth failure	±	++
Anatomy of involvement	Colon	Entire gut; rectal sparing
Histology and immunology		
Granuloma, stricture, fistula	Generally no	Yes
Transmural inflammation	No	Yes
Cytokines	Increased interleukin 4 and interleukin 5; normal γ interferon and interleukin 12	Normal interleukin 4 and interleukin 5; increased γ interferon and interleukin 12
Associated autoimmune diseases	Yes	No
Mucosal IgG subclass	IgG1	IgG2
Autoantibodies	+++	+
Management		
Response to antibiotics	No	Yes
Nutritional therapy	Not effective	Effective
Maintenance with 5-aminosalicylic acid	Effective	Small benefit
Ileoanal pouch	Yes	Generally no
Recurrence after surgery	No (apart from pouchitis)	Yes
Extraintestinal manifestations		
Sclerosing cholangitis	+++	+
Autoimmune hepatitis	+++	+

±=Equivocal association; +=weakly associated; ++=moderately well associated; +++ strongly associated.

Maintenance of remission

Since attacks recur, maintenance treatment is important. Sulphasalazine and the 5-aminosalicylic acid preparations are equally effective,[27] but the 5-aminosalicylic acid preparations are better tolerated. Intolerance to 5-aminosalicylic acid drugs occurs in about 10% of patients.[28] All 5-aminosalicylic acid preparations are potentially nephrotoxic and so regular monitoring of renal function is mandatory.[29] In patients with steroid resistant or dependent disease, immunosuppressive drugs such as azathioprine may maintain remission. Though the evidence supporting the use of azathioprine in ulcerative colitis is weaker than that in Crohn's disease, a recent survey confirmed its widespread use by British gastroenterologists.[30]

Medical treatment of severe disease

Corticosteroids are the mainstay of treatment. These may be given orally or intravenously, usually in a daily dose of 60-80 mg methylprednisolone intravenously, or 40-60 mg prednisolone orally. The dose and form of corticosteroids used are not fully backed up by dose ranging trials in severe disease. Where corticosteroids are ineffective several alternative treatments have been tried. Noticeable clinical improvement has been reported in patients treated with intravenous unfractionated heparin.[31,32] Cyclosporin, given intravenously (4 mg/kg)[33] or orally (4-9 mg/kg) has proved successful in inducing remission—for example, in a paediatric series 11 of 14 children responded.[34] Even lower doses may be effective, with fewer side effects. In many instances, however, colectomy is only delayed and not prevented—

seven of these cases needed colectomy within a year. The trend currently is to induce remission with cyclosporin and to maintain this with an immunosuppressive drug such as azathioprine. Over half of patients on this regimen may avoid colectomy in the longer term.[35] Avoiding colectomy, even for a short period, can be beneficial to some patients by providing time to think about surgery or allowing an elective rather than an emergency procedure. We believe that cyclosporin has a place in managing severe ulcerative colitis, a view supported by recent quality of life data.[36] Such treatment should, however, be confined to specialist centres.

The excellent results of, effectively curative, surgical treatment must always be taken into account when deciding whether to prolong medical treatment.

Failure of medical management and indications for surgery

Proctocolectomy or colectomy with rectal preservation may be an emergency procedure in fulminant colitis or toxic megacolon. More often, however, the procedure is elective after failure of medical treatment either through a lack of efficacy or unacceptable side effects (see fig D on website). Rarely, in a patient with long standing colitis, colectomy is necessary because of the development of severe dysplasia or carcinoma of the colonic epithelium. Until recently, surgical treatment implied permanent ileostomy, a prospect unacceptable to many patients. Now, advances in surgical technique have allowed the creation of an ileal reservoir or pouch, and with ileoanal anastomosis the need for permanent ileostomy is diminishing. Restorative surgery of this nature should be considered in every patient. Because of a child's small pelvis it may be technically advisable to delay pouch surgery until the mid teens, but often the best functional results after pouch surgery are seen in children.[37] Continent ileostomy has little use but may be considered in patients with existing conventional ileostomy or in pouch failures.

Ileal pouch-anal anastomosis and pouchitis

Restorative proctocolectomy with ileal reservoir has revolutionised surgery for ulcerative colitis, and with increasing confidence the indications have been widened to include those with more limited but refractory disease.[38] Pouchitis, a non-specific inflammation of the ileal reservoir, is the most frequent long term complication after ileal pouch-anal anastomosis for ulcerative colitis. This poorly understood condition may occur in up to one third of patients within 5 years of surgery, with two thirds of these having recurrent episodes.[39-41] Though faecal stasis is a popular explanation of the cause of pouchitis, no difference in the faecal concentrations of bacteria, bile acids, and short chain fatty acids has been reported between patients with or without pouchitis. Complex interactions between an immunologically susceptible mucosa, faecal stasis, and bacterial flora merit further investigation. Metronidazole is an effective first line treatment. In pregnant patients with ileal pouch-anal anastomosis, delivery by caesarean section should be considered as sphincter damage may be detrimental to pouch function. Though fertility is not reduced in ulcerative colitis in itself, postoperative fertility may be reduced after ileal pouch-anal anastomosis in women of childbearing age.[42]

Box 3: Risk factors for malignancy in ulcerative colitis

- Longstanding disease of more than 8 years
- Extensive colitis
- Sclerosing cholangitis
- Family history of colon cancer
- Expression of sialosyl-Tn antigen in mucosal biopsies
- ? Onset in childhood and adolescence

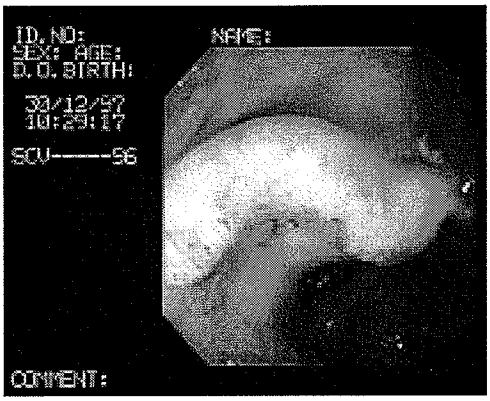

Fig 3 Highly dysplastic mass lesion in caecum of patient with ulcerative colitis of 12 years' duration

Malignancy complicating ulcerative colitis

Disease duration of more than eight years and disease extent proximal to the sigmoid colon are the two major determinants of increased risk of colorectal cancer in ulcerative colitis (box 3). Coexistent primary sclerosing cholangitis also increases the risk.[43] Screening for dysplasia by colonoscopy at regular intervals (1-2 years) remains the only feasible method for surveillance (fig 3). End points such as surgery must be understood by the patient before entering a surveillance programme. The limitations of colonoscopy as well as alternative options (colectomy after 10 years of extensive disease) need to be discussed in detail. Detection of dysplasia is an imperfect science and other reliable markers of a premalignant stage are needed. p53 mutations,[44] Ki-ras,[45] Ki-67, and sialosyl-Tn are among some of the candidates being evaluated.

Sclerosing cholangitis in ulcerative colitis

Primary sclerosing cholangitis is the commonest form of chronic liver disease associated with ulcerative colitis and may be present in 2-7% of patients depending on how diligently it is sought.[16] Primary sclerosing cholangitis associated with ulcerative colitis is twofold more common in men than women. The ulcerative colitis affects the entire colon in 90% of patients but symptoms are often mild. Endoscopic retrograde cholangiography is the best method of confirming the diagnosis, but magnetic resonance cholangiography is likely to become the non-invasive diagnostic method of choice. Perinuclear antineutrophil cytoplasmic antibodies may be detectable in serum.[17] Medical treatment

with agents such as corticosteroids, colchicine, penicillamine, and ursodeoxycholic acid has not been shown to retard progression of liver disease.[48] Endoscopic treatment may be beneficial in selected patients with dominant extrahepatic biliary strictures.[49] Progressive cholestatic jaundice with liver failure and development of cholangiocarcinoma are the two fatal consequences of primary sclerosing cholangitis. Orthotopic liver transplantation is the only treatment available to patients with advanced liver disease.[50] It is important to continue surveillance colonoscopy after liver transplantation to detect colonic dysplasia.[51]

Conclusion

The diagnosis and management of ulcerative colitis remain a challenge to clinicians. Rigorous epidemiological study of the negative relations between ulcerative colitis, smoking habit, and appendicectomy may yield further clues to the cause of this condition. Most patients can be managed wholly as outpatients. Symptomatic relapses are the rule, however, and so maintenance treatment with oral 5-aminosalicylic acid preparations is important to keep these to a minimum. Most have distal disease that is amenable to topical application of 5-aminosalicylic acid or corticosteroid preparations, and many patients will begin self treatment with these at the first signs of a flare up. The overall prognosis in ulcerative colitis is good, and with the exception of the first year of diagnosis when the risk of colectomy is statistically highest there is no significant excess in mortality.

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Exhibit K

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Inflammatory bowel disease: Sorting out the treatment options

ABSTRACT

An increasing array of treatments such as immunosuppressive drugs and tumor necrosis factor inhibitors can offer patients with ulcerative colitis and Crohn disease improved relief from symptoms with fewer adverse effects. Several additional drugs have shown promise, including nicotine, antimicrobials, and heparin.

KEY POINTS

First-line therapy for ulcerative colitis includes oral and rectal aminosalicylates for mild to moderate disease and steroids for moderate to severe disease. Steroids also are a preferred option for nonfistulizing Crohn disease.

Both azathioprine and 6-mercaptopurine have been shown to be safe and effective in ulcerative colitis and Crohn disease and enable adult and pediatric patients to avoid long-term use of corticosteroids.

Infliximab is an effective treatment for inflammatory and fistulizing Crohn disease.

PATIENTS WITH INFLAMMATORY bowel disease now have a variety of new treatment options. New uses are being found for old drugs, and new drugs are becoming available, including potent immunosuppressive drugs that control the unchecked inflammation of the gastrointestinal tract.

As internists and gastroenterologists, we need to be familiar with these new drugs as they become more widely used and as patients ask about new treatments they learn about via the Internet, television, and lay organizations. In this article, we offer an overview of current drugs and their indications for the treatment of ulcerative colitis and Crohn disease.

AMINOSALICYLATES

The aminosalicylates are among the oldest and most commonly used drugs for ulcerative colitis and Crohn disease.[1]

Sulfasalazine

Sulfasalazine, introduced in the 1940s for use in both inflammatory bowel disease and rheumatoid arthritis, is a compound of sulfapyridine and 5-aminosalicylic acid (5-ASA, mesalamine) linked by a diazo bond.

Action. Taken orally, the drug is delivered intact into the right colon and subsequently is degraded by colonic bacteria into 5-ASA (the active, anti-inflammatory moiety) and sulfapyridine, which helps transport 5-ASA to target areas.

Adverse effects. Although effective in treating ulcerative colitis and Crohn disease of the large colon, sulfasalazine is difficult to tolerate due to adverse effects in up to 50% of patients, principally due to the sulfapyridine moiety. The most common adverse effects are

TABLE 1

Aminosalicylates for the treatment of inflammatory bowel disease

MEDICATION	UNIT DOSE	DOSAGE	COLON ACTIVITY	SMALL BOWEL ACTIVITY
Sulfasalazine	500 mg	2–4 g/day	+++	–
Mesalamine (5-ASA)				
Asacol*	400 mg	1.6–4.8 g/day	+++	+
Balsalazide (Colazal)	750 mg	6.75 g/day	+++	–
Olsalazine (Dipentum)	250 mg	1–3 g/day	+++	–
Pentasa†	250 mg	2–4 g/day	++	++
Rowasa enema	4 g	4 g/day	+++	–
Rowasa suppository	1 g	2 g/day	+++	–

*Delayed-release tablet
†Controlled-release capsule

nausea, vomiting, anorexia, dyspepsia, malaise, and headaches, and these are especially troublesome at doses higher than 3 g per day. Rare, idiosyncratic reactions include fever, rash, hepatitis, pancreatitis, pneumonitis, and agranulocytosis. Patients with sulfa allergies should avoid sulfasalazine. Folate supplementation is recommended because sulfasalazine inhibits folate absorption.

All forms of 5-ASA are usually used only in colonic disease

Newer aminosalicylates

Newer aminosalicylates deliver 5-ASA to the distal bowel without the sulfapyridine, resulting in fewer adverse effects than sulfasalazine (TABLE 1, TABLE 2). These formulations are more expensive than sulfasalazine and, consequently, are generally prescribed if a trial of sulfasalazine fails.

Oral forms are indicated for treatment of mildly to moderately active ulcerative colitis, while the rectal forms are indicated for the treatment of active mild to moderate distal ulcerative colitis.

Pentasa (controlled-release capsule) consists of 5-ASA packaged in ethylcellulose microgranules that gradually release 5-ASA from the jejunum to the colon. It is approved only for ulcerative colitis but is often used for Crohn disease of the small bowel.

Asacol (delayed-release tablet), the most commonly used aminosalicylate, is 5-ASA enveloped in a coating that dissolves at a pH of 7 in the distal ileum and colon.

Rowasa, the enema and suppository forms of 5-ASA, is used for distal disease.

Olsalazine (Dipentum) is indicated for the maintenance of remission of ulcerative colitis in patients who cannot tolerate sulfasalazine. It consists of two 5-ASA molecules linked by a diazo bond and is activated only by bacterial cleavage. It is available in capsule form.

Balsalazide (Colazal) is indicated for the treatment of mildly to moderately active ulcerative colitis. It consists of 5-ASA molecules linked by a diazo bond to an inert, unabsorbed carrier molecule that is broken down by colonic bacteria.

Adverse effects. Mesalamine is well tolerated but can cause mild and transient headache and abdominal discomfort. Olsalazine causes secretory diarrhea in up to 10% of patients. Balsalazide can cause headache, abdominal pain, diarrhea, and nausea, but these are not usually severe enough to cause discontinuation of therapy. Rare side effects of 5-ASA drugs include pneumonitis, pericarditis, interstitial nephritis, and thrombocytopenia.

Recommendations for use

Whenever possible, sulfasalazine should be tried first due to its low cost.

Ulcerative colitis. Aminosalicylates are used in patients with mild to moderate disease activity or to maintain remission. For active disease, 5-ASA given both orally and topically is superior to either one alone in controlling

symptoms. Topical 5-ASA is superior to topical corticosteroids for distal disease.[2–6] 5-ASA suppositories (Rowasa 1 g) can be substituted in patients who cannot effectively retain enemas. For maintenance of remission in ulcerative colitis, all 5-ASA agents are similarly effective.

Crohn disease. All forms of 5-ASA have limited effectiveness in the small bowel and are usually used only in colonic disease. Pentasa has to be delivered at high doses, 4 g or 16 pills per day, to be effective in treating active disease of the small bowel.[7] These agents can be effective in maintaining remission in patients with ileitis or with surgically induced remission.[8]

STEROIDS

Steroids have been known since the early 1950s to be effective for both ulcerative colitis and Crohn disease.

Action

The mechanism of action of steroids is believed to be the blockade of phospholipase A_2 in the arachidonic acid cascade, causing an alteration in the delicate balance between the cytoprotective prostaglandins and proinflammatory leukotrienes.

Adverse effects

Prolonged use of steroids can cause osteoporosis, moon face, buffalo hump, impaired wound healing, hyperglycemia, and osteonecrosis of the femoral head.

Recommendations for use

Steroids are considered first-line therapy for patients with moderate to severe ulcerative colitis or nonfistulizing Crohn disease. The usual prednisone dosage is 40 mg per day with a taper over 2 to 4 months. For severe active disease, intravenous steroid regimens that provide the best response are hydrocortisone 100 mg every 8 hours or methylprednisolone 40 mg daily. Response to intravenous steroids is superior to that of equivalent oral doses.

Steroid analogues

Oral steroid analogues are locally active corticosteroids, designed to target areas of inflammation in the gastrointestinal tract before being inactivated by the liver during their first pass, thus avoiding some of the typical adverse effects of steroid use. Of the three steroid analogues tested in inflammato-

TABLE 2

Adverse effects of drugs commonly used for inflammatory bowel disease

Asacol
Headache, rash, nausea, diarrhea, abdominal pain, dry mouth, dysmenorrhea, anaphylaxis, hematologic effects, interstitial nephritis, hepatitis, myocarditis, peripheral neuropathy, Stevens-Johnson syndrome

Azathioprine or 6-mercaptopurine
Pancreatitis, leukopenia, bone marrow suppression, nausea, vomiting, diarrhea, rash, myalgia, alopecia

Cyclosporine
Seizures, leukopenia, thrombocytopenia, nephrotoxicity, susceptibility to infection, neoplasia, hypertension, hirsutism, tremor, gingival hyperplasia, paresthesias, nausea, vomiting, abdominal pain, diarrhea

Fish oils
Bad odor and taste

Infliximab
Susceptibility to infection, lupus-like syndrome, infusion reaction, lymphoma

Heparin
Hemorrhage, osteoporosis, thrombocytopenia

Methotrexate
Neurotoxicity, leukoencephalopathy, seizures, renal failure, leukopenia, pulmonary fibrosis, nausea, vomiting, photosensitivity, ecchymosis

Olsalazine
Secretory diarrhea, headache, rash, nausea, abdominal pain

Pentasa
Dysmenorrhea, anaphylaxis, hematologic effects, interstitial nephritis, hepatitis, myocarditis, peripheral neuropathy, Stevens-Johnson syndrome

Steroids
Adrenal insufficiency, osteoporosis, edema, appetite changes, mood changes, cushingoid features, avascular necrosis

Sulfasalazine
Headache, rash, nausea, diarrhea, abdominal pain, leukopenia, jaundice, fever, oligospermia, Stevens-Johnson syndrome, epidermal necrolysis, exfoliative dermatitis, agranulocytosis, hemolytic anemia

ry bowel disease, budesonide (Entocort EC) is the only one approved for use in the United States, and it is indicated specifically for mildly to moderately active Crohn disease involving the ileum or the ascending colon. Tixocortol and fluticasone are still undergoing testing.

Budesonide is designed to deliver steroid to the distal small bowel and proximal colon. Large randomized clinical trials[9–14] have shown that budesonide is more effective than placebo or 5-ASA in inducing remission by 8 weeks and nearly as effective as prednisolone in Crohn disease. Budesonide has fewer adverse effects than conventional steroids, but it is not free of adverse effects and is therefore not recommended for maintenance therapy. For left-sided ulcerative colitis, 2-g budesonide enemas (not available in the United States) are as effective as prednisolone enemas or 5-ASA therapy.[15,16]

AZATHIOPRINE AND 6-MERCAPTOPURINE

Budesonide has fewer ill effects than steroids, but it is not for maintenance therapy

6-Mercaptopurine (Purinethol) and azathioprine (Imuran) are immunosuppressive agents that can be used instead of long-term corticosteroid therapy.

Action

These drugs act by causing chromosomal breaks that blunt the proliferation of rapidly dividing cells such as lymphocytes. 6-Mercaptopurine is a purine analogue, and azathioprine is its S-imidazole precursor.

Adverse effects

Azathioprine was used as single-agent therapy in the large randomized clinical trial, the National Cooperative Crohn's Disease Study,[17] which compared azathioprine, prednisone, and sulfasalazine with placebo in treating active disease and in maintaining remission. The azathioprine arm was discontinued after only 17 weeks when three patients developed acute pancreatitis. Subsequently, fears of toxicity have led to azathioprine's fall from favor among gastroenterologists.

More recent studies have shown that these immunosuppressive drugs have a more favorable adverse effect profile than was originally believed. Fewer than 10% of patients stop therapy due to reversible bone marrow suppression, and fewer than 3% develop pancreatitis or allergy characterized by abdominal pain, fever, and rash.

Recommendations for use

Both azathioprine and 6-mercaptopurine have been shown to be safe and effective in ulcerative colitis and Crohn disease and enable adult and pediatric patients to avoid long-term use of corticosteroids.[18] 6-Mercaptopurine can be effective for closure of fistulas in Crohn disease.[19]

The duration of therapy most often required for effectiveness is at least 3 months, but delayed therapeutic responses have been demonstrated after 1 year of therapy. Because of the delayed response, the common practice of adjusting the dose according to the response may not be feasible. Some experts have suggested that the dose of immunosuppressive drug be increased until mild leukopenia (white blood cell count between 3,000 and 5,000 $\times$ 10^9/L) develops.[20] Patients treated to the point of leukopenia have a faster and more complete response.

Currently, monitoring of the metabolites 6-thioguanine nucleoside (the active metabolite) and 6-methylmercaptopurine (the metabolite associated with hepatotoxicity in some patients) can help the clinician find the very narrow window between effectiveness and toxicity of these drugs.[21]

CYCLOSPORINE

Cyclosporine is a cyclic polypeptide derived from either of two fungi and commonly used in conjunction with organ transplantation.

Action

Cyclosporine reversibly inhibits interleukin-2 (IL-2) gene transcription, causing a decrease in activity of cytotoxic T cells.

Adverse effects

Cyclosporine has significant adverse effects, which include nephrotoxicity, hepatotoxicity, hypertrichosis, gingival hyperplasia, tremors, paresthesias, seizures, and lymphoproliferative disorders.

Recommendations for use

Ulcerative colitis. Intravenous cyclosporine is useful for the treatment of severely active ulcerative colitis. In the only randomized clinical trial of cyclosporine use in ulcerative colitis,[22] all patients had severely active ulcerative colitis that failed to respond to intravenous corticosteroids. Cyclosporine was given at 4 mg/kg/day intravenously for no more than 7 days. The overwhelming success of cyclosporine (82%) compared with placebo (0%) forced an early termination of the trial. Five (45%) of the original 11 patients randomized to cyclosporine had a sustained response and did not require colectomy.

Experience with cyclosporine in clinical practice has not been as good[23,24]: only approximately 50% of patients had a response and avoided colectomy in the short term, and 33% avoided colectomy in the long term. This is most likely due to the inconsistent bioavailability of the oral form of cyclosporine.

Cyclosporine enemas are not effective in left-sided ulcerative colitis.[25]

Crohn disease. For Crohn disease, intravenous cyclosporine temporarily decreased drainage of fistulas and induced a remission in inflammatory disease.[26,27] Unfortunately, most patients have a relapse when cyclosporine therapy is converted to the oral form. Oral cyclosporine has been largely ineffective in treating inflammatory-type Crohn disease.[28,29]

Prophylaxis against *Pneumocystis carinii* pneumonia is advised in all patients taking cyclosporine.[30,31]

METHOTREXATE

Methotrexate, which was introduced in 1947 for the treatment of patients with acute leukemia, has been used in the treatment of chronic inflammatory disorders such as rheumatoid arthritis, psoriasis, asthma, primary sclerosing cholangitis, and primary biliary cirrhosis.

Action

Methotrexate binds to tetrahydrofolate reductase and interferes with purine synthesis of rapidly proliferating cells.

Adverse effects

Minor adverse effects such as nausea and abdominal cramps are due to folic acid antagonism by methotrexate and can be prevented with coadministration of folic acid 1 mg/day, without interfering with the effectiveness of the methotrexate.

Other adverse effects include hepatic fibrosis (when the total dose exceeds 1.5 g, with a lower threshold for obese and alcoholic patients), liver function test abnormalities, alopecia, pneumonitis, hypersensitivity, and teratogenicity. Due to the risk of teratogenicity, methotrexate is contraindicated during pregnancy, and women and men of reproductive age who take methotrexate should use birth control.

Clinical trial results

A small 12-week open-label trial of methotrexate 25 mg/week intramuscularly in Crohn disease patients[32] showed a 79% response rate, defined as lowering disease activity and prednisone use.

A randomized clinical trial of 141 patients[33] comparing the same dose of methotrexate with placebo over 16 weeks demonstrated a 39.4% rate of induced remission in the methotrexate group vs 19.1% in the placebo group. Seventeen percent of methotrexate-treated patients withdrew due to adverse effects, most commonly liver function test abnormalities and nausea.

Folic acid 1 mg/day prevents nausea and cramps due to methotrexate use

Another randomized clinical trial[34] in patients whose Crohn disease had responded to methotrexate showed that 15 mg/week of methotrexate was significantly better than placebo in maintaining remission.

Recommendations for use

Currently, methotrexate is used as long-term therapy in patients who do not respond to or cannot tolerate azathioprine or 6-mercaptopurine.

FISH OILS

Eicosapentaenoic acid (EPA, fish oil) is thought to suppress production of inflammatory agents, and its use has been suggested in the treatment of rheumatoid arthritis and psoriasis.

Action

Arachidonic acid metabolism produces leukotriene B_4, a potent proinflammatory cytokine. EPA in high doses interferes with arachidonic acid metabolism, leading to the production of the less-potent cytokine leukotriene B_5. High oral doses of fish oil also inhibit thromboxane A_2 production and thereby act as an anticoagulant.

Adverse effects

Unfortunately, the high doses of fish oils required in inflammatory bowel disease have a distasteful, odoriferous effect.

Clinical trial results

Crohn disease. In a study of Crohn disease patients who were in remission but were at high risk of relapse,[35] 28% of those who took 2.7 g of EPA daily had a relapse by 12 months vs 69% of placebo-treated patients. Diarrhea was the principal adverse effect requiring discontinuation of treatment. These results, though, could not be confirmed subsequently.[36]

Ulcerative colitis. In a study of the effects of fish oil supplementation in moderately active ulcerative colitis,[37] 18 patients taking prednisone and sulfasalazine took 18 capsules per day of fish oil (equal to 3.24 g/day of EPA and 2.16 g/day of docosahexaenoic acid) for 4 months, followed by a 1-month "washout" period, then 4 months of 18 placebo capsules. The investigators observed that fish oil supplementation allowed steroids to be tapered significantly.[37]

Bad taste and odor limit use of fish oil

In another study,[38] more than 5 g of EPA per day delayed relapse for 3 months compared with placebo, but the rate of relapse was similar at 2 years, leading the authors to conclude that fish oil supplementation can "temporarily retard, but not prevent," relapse in patients with ulcerative colitis.[38]

Recommendations for use

Even though there are some promising studies, the lack of long-term effectiveness and the bad taste make this a medicine not actively being used in clinical settings.

INFLIXIMAB

Infliximab (Remicade) is a chimeric mouse-human monoclonal antibody approved for the treatment of inflammatory Crohn disease (one 5-mg/kg infusion over 2 hours) and fistulous Crohn disease (three 5-mg/kg infusions over 6 weeks).

Action

Elevations of tumor necrosis factor-alpha in the stool of patients with Crohn disease are associated with an increase in disease activity. A single intravenous infusion of infliximab induces lysis of mononuclear cells, thereby causing a decrease in all of the inflammatory cytokines that they produce, including tumor necrosis factor-alpha.[39,40]

Adverse effects

Adverse effects of infliximab include upper-respiratory infections, abdominal pain, fatigue, myalgia, infusion reactions, nausea, and the development of both anti-ds-DNA antibodies and anti-chimeric antibodies. The development of lymphoma has been reported in patients receiving infliximab infusions,[41–43] but the incidence is very low, and more data are needed to better estimate the risk of lymphoma in these patients.[44]

Clinical trial results

In one clinical trial of patients with inflammatory Crohn disease,[45] infliximab induced a clinical response in 81% of patients taking the drug vs 17% in placebo-treated patients; it induced clinical remission in 33% vs 4% of placebo-treated patients. Adverse effects were minor, short-lived, and no different than in placebo-treated patients.

In a study of Crohn disease patients with enterocutaneous or perianal fistulas,[46] infliximab induced a response in 68% of patients vs 26% in the placebo group and induced complete fistula closure in 55% vs 13% in the placebo group; these fistulas remained closed for a median of 3 months.[46] Adverse effects in treated patients were no different than in the placebo group.

Maintenance therapy with infliximab given every 8 weeks for four additional doses was more effective than placebo,[47] but maintenance therapy with infliximab has not been approved.

Recommendations for use

Infliximab is an effective treatment for both inflammatory and fistulizing Crohn disease.

HEPARIN

Interest in heparin in the treatment of inflammatory bowel disease was fueled by interesting case reports of unexpected side effects of heparin used in patients with other conditions.

A patient with active ulcerative colitis who required heparin for deep venous thrombosis experienced a complete remission of his colitis after heparin administration.[48] Eight of 9 additional patients with active ulcerative colitis (and no deep vein thrombosis) refractory to corticosteroids who were treated with 10,000 units of heparin subcutaneously twice daily showed a complete remission; the other patient showed a partial response.

Heparin also has been shown to induce a rapid resolution of chronically active symptoms of diarrhea, hematochezia, arthralgia, and pyoderma gangrenosum in a patient with ulcerative colitis.[49]

Unfractionated heparin given to patients with either ulcerative colitis or Crohn disease had a beneficial effect in ulcerative colitis patients only.[50] Seven of 13 ulcerative colitis patients achieved complete remission within 4 weeks, but one patient had massive lower gastrointestinal bleeding.

In another series, 12 of 16 steroid-resistant ulcerative colitis patients given heparin had a marked improvement in symptoms within 1 week.[51]

Action and recommendations

Such findings suggest that the pathogenesis of ulcerative colitis may involve microthrombosis in the intestinal circulation, which is resolved by heparin. While these reports are encouraging, further studies are warranted to determine what utility heparin will have for steroid-refractory ulcerative colitis.

ANTIMICROBIALS

Investigators have long postulated that a transient infection may initiate a cascade of inflammatory events that may lead to ulcerative colitis in predisposed individuals.

Ciprofloxacin

The literature contains conflicting reports about the use of ciprofloxacin (Cipro) in inducing remission in such patients. For example, for an acute flare-up of disease, ciprofloxacin in addition to prednisolone and 5-ASA had no additional beneficial effect when compared with placebo.[52] But 6 months of maintenance therapy with ciprofloxacin in ulcerative colitis patients with a medically induced remission was associated with a relapse rate of only 21% at 6 months vs 44% in placebo-treated patients ($P < .02$).[53]

Metronidazole

Metronidazole (Flagyl) has been shown to be effective in active colonic Crohn disease. Some benefit can be derived from adding metronidazole in Crohn disease patients whose disease is only partially responsive to 5-ASA or to steroids. Metronidazole also has beneficial activity in treating perianal complications in Crohn disease patients.

Both metronidazole and ciprofloxacin have benefit in certain clinical situations, eg, in the treatment of acute pouchitis in ulcerative colitis patients following proctocolectomy and ileal pouch-anal anastomosis.

Adverse effects

Adverse effects of metronidazole include peripheral neuropathy, neutropenia, nausea, vomiting, and a metallic taste. Adverse effects of ciprofloxacin include nausea, diarrhea, vomiting, abdominal pain, and headache in up to 10% of patients.

Evidence of heparin benefit in ulcerative colitis needs more study

Recommendations for use

Although these antibiotics are useful in the treatment of pouchitis, antibiotics currently have no role in the therapy of ulcerative colitis or inflammatory Crohn disease. Although ciprofloxacin is more expensive than metronidazole, it is frequently substituted if patients cannot tolerate metronidazole.

NICOTINE

Intriguing case reports of improvements in ulcerative colitis disease activity with the intake of nicotine from cigarettes or nicotine gum led to equally intriguing epidemiologic studies. The consistent finding of these studies was that cigarette smoking protected against the development of ulcerative colitis.[54,55] At

diagnosis, fewer than 15% of adults with ulcerative colitis smoke vs approximately 35% of the general US adult population.

Action

The cellular mechanism for this effect is not known, but cigarette smoking is associated with global suppression of the immune system, as well as potentiation of the protective mucus barrier in the colon.

Clinical trial observations

Ulcerative colitis patients with moderately active disease often improve with nicotine intake. Nicotine gum, up to 10 squares or 20 mg/day, is effective and is best tolerated among former smokers.[56] The nicotine patch (up to 25 mg/day) is a delivery system that minimizes side effects and has also been found effective.[57,58] Nicotine patches are not effective for maintenance, and using them for more than 8 weeks risks addiction.[59]

Adverse effects

Adverse effects include parched throat, tachycardia, headache, and nausea.

Moderately active ulcerative colitis often improves with nicotine intake

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Exhibit L

Diagnosis and Treatment of Pouchitis

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Keywords

Ileal pouch, inflammatory bowel disease, restorative proctocolectomy

Abstract: Ileal pouch-anal anastomosis following total proctocolectomy has become part of the standard surgical treatment for patients with ulcerative colitis or familial adenomatous polyposis who require colectomy. Although this surgery has improved patient quality of life and significantly reduced the risk of dysplasia or neoplasia in ulcerative colitis patients, complications are common. Pouchitis is the most common long-term complication of ileal pouch surgery and has a significant adverse impact on patient quality of life. The diagnosis and differential diagnosis of pouchitis are not straightforward, and the management of pouchitis, particularly chronic antibiotic-refractory pouchitis, which is one of the leading causes of pouch failures, can be challenging.

The last decade has witnessed major advances in medical treatment of ulcerative colitis (UC). The options for medical therapy of moderate-to-severe UC have extended to anti-tumor necrosis factor (TNF)-α biologic regimens. However, it is not clear whether these new agents will ultimately alter the natural history of UC. Approximately 30% of patients with UC eventually require colectomy.[1] Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) has become the surgical treatment of choice for the majority of patients with UC who fail medical therapy or develop dysplasia and the majority of patients with familial adenomatous polyposis (FAP). The advantages of the surgical procedure include re-establishment of gastrointestinal continuity; improvement in health-related quality of life; positive impact on body image; avoidance of long-term use of UC-related medications in the majority of patients; and a substantial reduction in the risk of dysplasia or cancer. However, after the surgery, adverse outcomes or complications often occur, of which pouchitis is the most common long-term inflammatory complication.

Prevalence

Pouchitis is generally considered a nonspecific inflammatory condition in the ileal pouch reservoir.[2] Reported cumulative frequency rates of pouchitis 10 years after IPAA surgery range from 23% to 46%.[3,4] It is estimated that approximately 50% of patients who undergo IPAA surgery for UC will develop at least 1 episode of

pouchitis.[5] The estimated incidence of pouchitis within 12 months of ileostomy take-down has been reported to be as high as 40% in one drug trial.[6] The discrepancy in the reported cumulative frequencies from different institutions likely results from the variance in diagnostic criteria, the intensity of follow-up with or without pouch endoscopy, and the inclusion or exclusion of other inflammatory or functional disorders of the pouch and related surgical conditions.

Pathophysiology

Pouchitis almost exclusively occurs in patients with underlying UC or indeterminate colitis and is rarely seen in patients with FAP.[7,8] Although the etiology and pathogenesis of pouchitis are not entirely clear, the alteration in bowel anatomy from the surgery may create an "inflammation-prone" environment. The normal function of the distal ileum, which involves the absorption of nutrients, is artificially converted to that of a storage reservoir. Qualitative and quantitative changes in the ileal pouch may constitute a triggering factor for the development of pouchitis.[9-12] Evidence suggests that an abnormal mucosal immune response to altered microflora in the pouch leads to acute and/or chronic inflammation.[6,12-15] Immune mechanisms of pouchitis have been extensively studied in a fashion similar to those of inflammatory bowel disease. An ex-vivo study has demonstrated that an ileal pouch of long duration has increased bacterial permeability.[16-21] Proinflammatory cytokines such as TNF-α are released mainly in inflamed mucosa by macrophages and monocytes, leading to tissue injury, and are considered a secondary pathophysiologic mechanism in pouchitis.[21] As in UC, the production of other inflammatory mediators, including cytokines,[22-25] cell adhesion molecules,[26] platelet-activating factor,[27] lipoxygenase products of arachidonic acids,[28] and proinflammatory neuropeptides[23,29-31] is also increased. Abnormalities of immunoregulatory cytokines such as interleukin (IL)-2, interferon-gamma,[19,32] IL-4,[32] and IL-10 are also seen in pouchitis. Imbalance between proinflammatory and immunoregulatory cytokines has been described in patients with pouchitis.[25] However, it is likely that those abnormalities in mucosal immunity are nonspecific and secondary in nature.

Risk Factors

The risk factors associated with pouchitis have been extensively studied. Genetic polymorphisms such as those associated with the IL-1 receptor antagonist[33-35] and *NOD2/CARD15*[36] may increase the risk of pouchitis. The reported risk factors of pouchitis also include noncarrier status of TNF allele 2,[35] extensive UC,[4,37,38] backwash ileitis,[37] proctocolectomy thrombocytosis,[39] concurrent primary sclerosing cholangitis,[3,40,41] seropositive perinuclear antineutrophil cytoplasmic antibodies (pANCA),[42,43] being a nonsmoker,[38,44] and use of nonsteroidal anti-inflammatory drugs (NSAID).[38,44] In addition to pANCA, the presence of the serologic markers *anti-Saccharomyces cerevisiae* antibodies (ASCA), the Crohn's disease (CD)-related antigen from *Pseudomonas fluorescens,* and the outer membrane porin C of *Escherichia coli* in patients with pre-operative indeterminate colitis appears to be associated with persistent inflammation of the pouch after restorative proctocolectomy.[45] Acute and chronic pouchitis may be associated with different risk factors.[38,46]

Presentation

Patients with pouchitis can develop a wide range of clinical presentations, including increased stool frequency, urgency, tenesmus, incontinence, nocturnal seepage, abdominal cramping, and pelvic discomfort. Although bloody bowel movements are uncommon in typical pouchitis, patients with IPAA with or without pouchitis can have iron-deficiency anemia.[47,48] Patients with severe pouchitis occasionally present with fever, dehydration, and malnutrition, which may require hospitalization. Patients may chiefly complain of predominantly extraintestinal symptoms such as arthralgia. These symptoms, however, can be present in other disorders of the pouch, including cuffitis, CD of the pouch, proximal small-bowel bacterial overgrowth, and irritable pouch syndrome.

Diagnosis

The diagnosis of pouchitis should not depend solely upon the presenting symptoms of a patient. The severity of symptoms does not necessarily correlate with the degree of endoscopic or histologic inflammation of the pouch.[49,50] A combined assessment of symptoms and endoscopic and histologic features is ideal for the diagnosis and differential diagnosis of pouchitis. There are no universally accepted diagnostic criteria for pouchitis. The 18-point Pouchitis Disease Activity Index (PDAI), although the most commonly used index in clinical trials, is seldom utilized in routine clinical practice.[51]

Pouch endoscopy yields valuable information on the severity and extent of mucosal inflammation (Figure 1), the presence or absence of concurrent backwash ileitis, CD of the pouch (Figure 2) or cuffitis (Figure 3), and the presence or absence of structural abnormalities such as strictures, sinus openings, and fistula openings. In addition, pouch endoscopy with segmental biopsy is the main surveillance procedure for dysplasia and can deliver

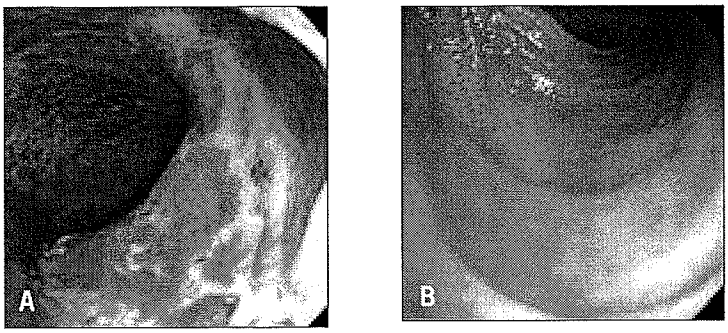

Figure 1. Active pouchitis: Diffuse endoscopic inflammation of the pouch (A) with normal afferent limb mucosa (B).

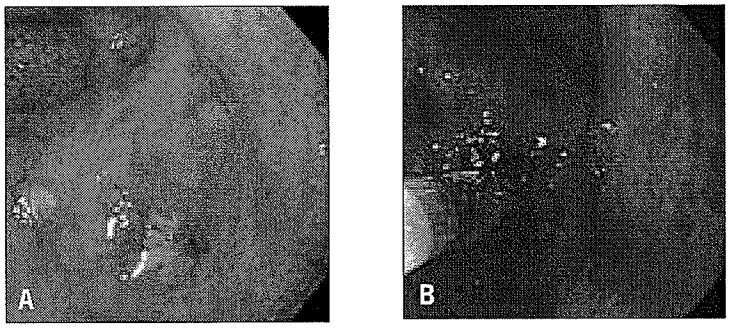

Figure 2. Crohn's disease of the pouch: Pouch inlet stricture (A) with balloon dilation therapy (B).

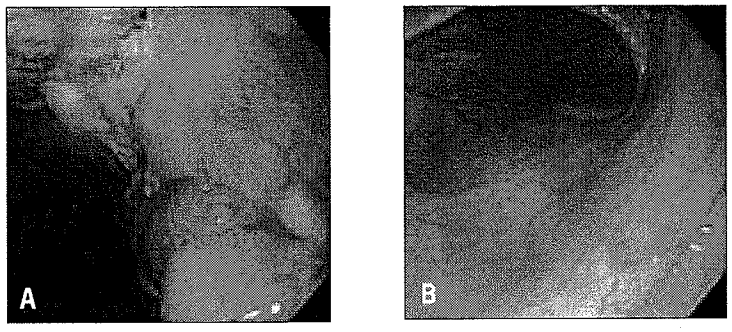

Figure 3. Cuffitis: Inflammation at the anal transitional zone or cuff (A) with normal pouch mucosa (B).

effective therapy, including balloon stricture dilations and polypectomy. Histopathology is invaluable for the detection of dysplasia or neoplasia, viral inclusion bodies of cytomegalovirus infection, granulomas, pyloric gland metaplasia, mucosal prolapse, and ischemic changes. It should be noted that villous blunting and an increase in the number of mononuclear cells in the lamina propria can be part of the "normal" adaptive changes of the pouch mucosa to fecal stasis in the pouch and do not necessarily indicate pouchitis or CD of the pouch.

In cases of suspected complicated pouchitis, CD of the pouch and complications related to surgical procedures should be excluded. Imaging studies such as contrast pouchography, computed tomography, and particularly magnetic resonance imaging of the pelvis are typically utilized to assess the presence of mucosal and transmural disease activity within and around the pouch.[52] Wireless capsule endoscopy appears to be safe for use in patients with chronic pouchitis[53] or anemia[54] for assessment of small-bowel diseases.

Disease Variance

The disease course of pouchitis varies. Pouchitis likely represents a disease spectrum from acute, antibiotic-responsive to chronic, antibiotic-refractory. Based upon

various criteria, pouchitis can be classified into the following categories: idiopathic versus secondary (based upon etiology); remission versus active (based upon disease status); acute versus chronic (based upon disease duration); infrequent episodes versus relapsing disease versus continuous disease (based upon disease pattern); and responsive versus refractory (based upon response to antibiotic therapy).[55] Although the majority of patients with pouchitis respond favorably to antibiotic therapy, particularly in the initial stages of disease, some patients develop pouchitis refractory to routine antibiotic treatment. This leads to an additional useful clinical classification based upon the response to antibiotic therapy.[56] Analogous to the classification of UC according to response to or dependency on corticosteroids, pouchitis can be classified based upon the manner of the patient's response to antibiotics: antibiotic-responsive, antibiotic-dependent, or antibiotic-refractory pouchitis.[44] A subpopulation of patients experience pouchitis associated with identifiable and modifiable causes (namely secondary pouchitis), such as *Clostridium difficile*[57,58] and cytomegalovirus[59,60] infections as well as regular NSAID use.[61] A recent study using immunohistochemistry and polymerase chain reaction found that viral genes and proteins were detected in samples from 12 of 34 patients (35.2%) with pouches, more frequently in patients with pouchitis than those without pouchitis. Cytomegalovirus infection may contribute to the disease course of pouchitis in some patients,[62] though whether antiviral therapy is beneficial is not clear.

Disease Management

As the majority of patients who develop acute pouchitis do so within the first year post-IPAA,[63] the probiotic VSL#3, which contains viable lyophilized bacteria with 4 strains of *Lactobacillus*, 3 strains of *Bifidobacterium* species, and *Streptococcus salivarius* subsp. *Thermophilus*, was evaluated for the primary prophylaxis of the initial pouchitis episode. Two of 20 patients (10%) treated with VSL#3 developed pouchitis within 12 months after IPAA, whereas 8 of 20 patients (40%) experienced pouchitis in the placebo group during the same period of time.[6]

Management and prognosis vary among the different types of pouchitis. For antibiotic-responsive pouchitis, first-line therapy includes a 14-day course of metronidazole (15–20 mg/kg/day) or ciprofloxacin (1,000 mg/day).[64,65] A randomized trial of ciprofloxacin and metronidazole showed that patients treated with ciprofloxacin experienced significantly greater reductions in PDAI scores and fewer adverse effects than those treated with metronidazole.[65] A small randomized trial of oral rifaximin 1,200 mg daily versus placebo (N=18) showed a marginal therapeutic benefit for active pouchitis.[66] Other agents used in open-label trials include tetracycline, clarithromycin, amoxicillin/clavulanic acid, doxycycline, rifaximin, budesonide enemas,[67] alicaforsen enemas, an antisense inhibitor of intercellular adhesion molecule-1,[68] and AST-120, a highly adsorptive, porous, carbon microsphere.[69]

Patients with antibiotic-dependent pouchitis often require long-term maintenance therapy with either antibiotics or probiotics for maintenance of disease remission. A randomized trial of VSL#3 at a dose of 6 g daily was conducted for the maintenance and secondary prophylaxis of pouchitis relapse after remission was induced by oral ciprofloxacin (1,000 mg/d) and rifaximin (2,000 mg/d). During the 9-month trial in 40 patients with relapsing pouchitis, only 15% of the probiotic group relapsed as opposed to 100% of the placebo group.[14] A separate randomized trial of VSL#3 in patients with antibiotic-dependent pouchitis showed that 17 of 20 patients (85%) in the VSL#3 group maintained clinical remission compared to 1 of 16 patients (6%) in the placebo group.[15] However, in a recent postmarket, open-label trial of VSL#3 in 31 patients with antibiotic-dependent pouchitis, patients received 2 weeks of treatment with ciprofloxacin followed by VSL#3.[70] After 8 months, 6 of the 31 patients (19%) were still taking VSL#3 and the remaining 25 patients (81%) had stopped mainly due to the lack of efficacy, low compliance, or the development of adverse effects.[70] A small open-label trial of high-dose VSL#3 showed that treatment with the agent resulted in remission in 16 of 23 patients (69%) with active pouchitis.[71] However, the role of probiotics in induction therapy warrants further study.

Antibiotic-refractory pouchitis is often difficult to treat and a common cause of pouch failure. Patients typically do not respond to full-dose, single-agent antibiotic therapy. It is important to investigate contributing causes (in secondary pouchitis) related to failure of antibiotic therapy. Secondary causes of refractory disease include the use of NSAIDs, concurrent *C. difficile* or cytomegalovirus infection, celiac disease and other autoimmune disorders, cuffitis, CD of the pouch, pouch ischemia, and inflammatory polyps of the pouch.[72] There have been no randomized trials in the literature for this category of pouchitis. For patients without obvious causes of pouchitis, treatment options include a prolonged course of combined antibiotic therapy, 5-aminosalicylates, corticosteroids, immunosuppressive agents, or even biologic therapy. Regimens reported to be safe and effective in open-label trials include ciprofloxacin (1,000 mg/d) combined with one of the following: rifaximin (2,000 mg/d),[73,74] metronidazole (1,000 mg/d),[75] or tinidazole (1,000–1,500 mg/d)[76] for 4 weeks. However, maintenance of remission in this group of patients after induction therapy with dual antibiotics remains a challenge.[77] In addition, overuse of

antibiotics may explain the possibility that the microflora responsible for pouchitis may shift from conventional to nonconventional forms, such as *C. difficile*,[58] fungi,[78] or even parasites (the authors' nonpublished data). Anti-inflammatory agents, immunomodulators, and biologic therapy have been used to treat pouchitis; these agents include bismuth carbomer enemas, short-chain fatty acid enemas, glutamine enemas, mesalamine enemas, oral budesonide,[79] 6-mercaptopurine, and infliximab.

Natural History and Prognosis

The natural history of pouchitis is poorly defined. In a study of 100 consecutive UC patients who underwent restorative proctocolectomy with IPAA, 32 patients developed pouchitis episodes and 5 patients had chronic refractory pouchitis, 2 of whom had pouch.[55] Few studies have been conducted to identify the natural history of pouch and pouchitis. Patients with initial episodes of pouchitis almost uniformly respond to antibiotic therapy. However, pouchitis relapse is common. Of the patients with acute pouchitis, 39% have a single acute episode that responds to antibiotic therapy whereas the remaining 61% of patients develop at least 1 recurrence.[80] Approximately 5–19% of patients with acute pouchitis develop refractory or rapidly relapsing forms of the disease.[81-83] The disease course of antibiotic-responsive pouchitis may evolve into antibiotic-dependent pouchitis and then antibiotic-refractory pouchitis. The latter is one of the leading causes for pouch failure, resulting in pouch excision or permanent diversion. Although concurrent primary sclerosing cholangitis appears to be a risk factor for pouchitis,[3,40,41] liver transplantation with posttransplant use of immunosuppressive agents does not appear to have an adverse impact on the disease course of pouchitis.[84,85] In addition, chronic inflammation of the pouch and cuff may convey an increased risk for the development of dysplasia or cancer.[86,87]

Summary

Pouchitis is the most common long-term complication of restorative proctocolectomy. Its natural history, however, has not yet been defined. Patients with pouchitis can have a wide range of clinical presentations, disease courses, and prognoses. As medical therapy for pouchitis is largely antibiotic-based, management of antibiotic-dependent and antibiotic-refractory pouchitis remains a challenge.

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Exhibit M

Gut 1993; 34: 1539–1542

Refractory pouchitis: does it reflect underlying Crohn's disease?

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Abstract

Typical 'pouchitis' is a well recognised complication of ileal pouches in ulcerative colitis. Infrequently, a refractory pouchitis (RP) presents with certain clinical, endoscopic, and pathological features resembling Crohn's disease and is often ascribed to misdiagnosis of the initial colitis. To test that hypothesis and to identify risk factors for RP, this study reviewed cases of presumed ulcerative colitis with ileal pouches constructed at The Mount Sinai Hospital between 1973 and 1986. Twenty four cases with RP (16 Kock pouches and eight pelvic pouches) and 21 controls were compared for eight clinical variables. The original colectomy slides from 15 RP and 18 control cases were reviewed blindly, classified into five histological categories (corresponding to definite ulcerative colitis, definite Crohn's disease, and three indeterminate groups), and scored for 23 histological features. There were no significant clinical differences between RP and control cases except for more frequent extraintestinal manifestations (38% *v* 5%) and male preponderance (79% *v* 43%) in RP. There were also no significant differences between the distributions of RP cases and controls among the five histological categories or in the 23 histological features studied. Refractory pouchitis therefore does not seem to reflect underlying Crohn's disease, but may be linked to immunological mechanisms that are manifested clinically as extraintestinal complications.

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Florid active mucosal inflammation with disturbed bowel function, often called 'pouchitis', is a comparatively common complication of ileal reservoirs created after colectomy, usually for ulcerative colitis, occurring in roughly 20% of cases (range 7–42%).[1–4]

Pouchitis usually responds well to treatment with antibiotics, but in a small subset of cases the inflammation is clinically severe, endoscopically atypical, and relatively refractory to conventional treatment. These cases seem to represent a distinct inflammatory condition, which we have termed 'refractory pouchitis' (RP). Because the endoscopical features of RP are not dissimilar to those of Crohn's ileitis, RP has often been attributed to underlying Crohn's disease, presumably 'misdiagnosed' as ulcerative colitis pre and perioperatively. To test the validity of this notion and to identify potential risk factors for RP, we reviewed our cases of RP at The Mount Sinai Hospital and compared the pathological features of their colectomy specimens as well as their clinical features to those of a control group without RP.

TABLE I *Endoscopic lesions in refractory pouchitis*

Lesions	*No of patients*
Aphthous ulcers	6
Serpiginous ulcers	8
Ileitis proximal to reservoir	14
Cobblestone mucosa	4

Patients

All cases were selected from ileal pouches constructed between 1973 and 1986 at The Mount Sinai Hospital.

Refractory pouchitis – We selected 24 cases that were well known to their gastroenterologists and that fit the clinical, endoscopical, and pathological criteria for RP as adopted at an International Workshop on Pouchitis in London in January 1989,[5] including clinical symptoms (bloody diarrhoea, malaise, or weight loss, or all three); resistance to treatment with antibiotics and steroids; and endoscopical features (serpiginous ulcerations, cobblestone mucosa, or ileitis proximal to the reservoir, or all three).[6–8] Table I shows the frequency of each of the defining endoscopic lesions; in some cases, of course, more than one feature was present. Biopsies of the pouches of all these patients were reported as showing active chronic inflammation. The mean duration between pouch construction and onset of pouchitis was 22·3 months (range 2–72 months). Four of the 24 RP patients ultimately required pouch removal and conversion to a standard Brooke ileostomy.

Controls comprised 21 patients with ileal pouches constructed during the same period who had been followed up for a mean duration of 43 months (range 15–119 months) and who had had endoscopies that showed no evidence of active inflammation. Table II shows the distribution of Kock pouches (continent ileostomies) and pelvic pouches between RP cases and controls.

The medical records of all cases were reviewed for the following eight clinical features: sex, age at onset of colitis, age at colectomy, indications for colectomy, age at time of pouch construction, extraintestinal manifestations before colectomy,

TABLE II *Distribution of pouches in the group*

Type of pouch	*RP (n=24)*	*Controls (n=21)*
1 Kock	16	16
2 Ileoanal or pelvic	8	5

RP=refractory pouchitis.

TABLE III *Histological classification of colectomy specimens*

Category	*Criteria*
A	Definite UC; diffuse, continuous proctocolitis limited to mucosa and superficial submucosa.
B	Highly suggestive of UC, but focally evincing one of several histological features more commonly associated with CD than UC, for example, cleft like mucosal ulceration, neuronal hyperplasia, intramural lymphoid aggregates beneath intact mucosa, submucosal lymphangiectasia; also fulminant colitis, even if lacking any of these features.
C	Indeterminate colitis, with more than one, or increased prominence, of the histological features indicated for category B; also fulminant colitis with any one of these features.
D	Indeterminate colitis, suggestive of CD, with one or more histological features considered characteristic of CD, for example, skip segments, focality of inflammation, fissuring ulcerations, focal chronic ileitis with pseudopyloric metaplasia; no diagnostic granulomata.
E	Definite CD, with typical granulomata in an appropriate histological setting.

UC=ulcerative colitis, CD=Crohn's disease.

use of steroids before colectomy, and immediate postcolectomy complications.

Methods

Microscopic slides of 33 resection specimens were available for review (31 from The Mount Sinai Hospital and two from other hospitals), comprising 15 patients with RP and 18 controls. The specimens consisted of 21 total colectomies (several performed in two stages), 11 subtotal colectomies, and one second stage proctosigmoidectomy for which the corresponding colectomy specimen was not available. The original pathological diagnoses were either ulcerative colitis or indeterminate. The original macroscopic descriptions were reviewed to ensure that the available slides represented an adequate sampling of significant pathology. All slides were then reviewed by one pathologist (NH), who was aware that the cases comprised both RP and control groups but who had not seen the cases previously and had no knowledge of individual clinical histories or outcomes. An average of 15 sections (range 7–32) were available for both total and subtotal colectomies and six sections for the single proctosigmoidectomy, not counting sections of lymph nodes and stomas. Gross photographs of specimens were not examined, as they were not available for all cases.

Based on the microscopic review, each case was assigned to one of five histological categories: cases representing definite ulcerative colitis were assigned to category A, cases representing definite Crohn's disease to category E, and cases containing overlapping features to indefinite categories B, C, or D, depending on the prominence and diagnostic significance of these features (Table III). Each case was also evaluated and graded for 23 individual histological features (Table IV), mostly those considered useful in the differential diagnosis of ulcerative colitis and Crohn's disease, but including others that were added in the search for histological features that might distinguish RP from controls.

TABLE IV *Individual histological features*

Left to right gradient of chronic colitis (+/−)
Skipped segments (+/−)
Chronic ileitis (+/−)
Anal inflammation (+/−)
Microscopic focal inflammation (+/−)
Aphthous ulcers (+/−)
Sarcoid like granulomata (+/−)
Maximum depth of chronic inflammatory infiltrates beneath intact mucosa*
Fissures: frequency (0 to 3+), depth*
Ulcers: frequency (0 to 3+), depth*
Lymphoid aggregates: frequency (0 to 3+), depth*
Activity of mucosal inflammation (0 to 3+)
Thickening and splaying of muscularis mucosa (0 to 3+)
Paneth cell metaplasia (0 to 3+)
Neuronal hyperplasia (0 to 3+)
Mucus secretion (0 to 3+)
Hyperemia (0 to 3+)
Submucosal lymphangiectasia (0 to 3+)
Submucosal fibrosis (0 to 3+)
Submucosal fat (0 to 3+)
Submucosal vascularity (0 to 3+)
Follicular proctitis (0 to 3+)
Rectal crypt atrophy (0 to 3+)

*Maximum depths of fissures, ulcers, chronic inflammatory infiltrates, and lymphoid aggregates graded as follows: superficial, mid, or deep submucosa; superficial or deep muscularis propria; serosa.

TABLE V *Precolectomy clinical features*

	RP (n=24)	*Kock (n=16)*	*Pelvic (n=8)*	*Controls (n=21)*
Sex distribution (M:F)*	19:5	12:4	7:1	9:12
Mean age of patient (y)	36·7	36·9	36·3	37·5
Mean age of onset of UC (y)	19·6	17·7	23·5	19·5
Mean age at colectomy (y)	27·5	26·1	30·1	29·5
Mean duration between onset of UC and colectomy (y)	7·9	8·4	6·7	10·4
Mean age at time of pouch construction (y)	29·2	28·4	30·8	31·1
Use of steroids before colectomy	24	16	8	20
Presence of EIMs before colectomy*	9	6	3	1

*Statistically significant differences between RP and controls.
RP=refractory pouchitis, UC=ulcerative colitis, EIMs=extraintestinal manifestations.

Differences in the sex distribution and in the frequency of precolectomy extraintestinal manifestations were analysed by the χ^2 test and the remaining clinical variables by the Student t test. An association of each of the histological features with the presence or absence of pouchitis was sought by the χ^2 test.

Results

Table V shows the comparison of clinical features between RP cases and controls. There was no statistically significant difference between the two groups except for sex distribution and frequency of precolectomy extraintestinal manifestations. Refractory pouchitis patients showed a male preponderance of 79% *v* 43% in the control cases ($p<0·03$). They also had more prevalent precolectomy extraintestinal manifestations of 38% (9/24) *v* 5% (1/21) in the controls ($p<0·025$). These clinical profiles of RP cases did not differ significantly between Kock and pelvic pouches (Table V). Table VI shows the specific extraintestinal manifestations in the RP and control groups. In three of the RP cases, but in none of the controls, extraintestinal manifestations had provided the principal indication

TABLE VI *Distribution of extraintestinal manifestations (EIMs)*

EIMs	*RP (n=24)*	*Controls (n=21)*
Erythema nodosum alone	5	1
Erythema nodosum+arthritis	1	0
Pyoderma gangrenosum alone	1	0
Pyoderma gangrenosum+arthritis	1	0
Arthritis alone	1	0
Total	9	1

TABLE VII *Indications for colectomy and perioperative features*

	RP cases (n=21)	*Controls (n=21)*
Intractability	13	13
Bleeding	3	4
EIMs	3	0
Perforation	1	1
Dysplasia	1	1
Toxic megacolon	2	2
Growth failure	1	0
Emergency colectomy	5	4
Elective colectomy	19	17
Immediate postoperative complications	1	2

Abbreviations the same as in Table V.

TABLE VIII *Histological classification of resected colons*

Category	*RP (n=15)*	*Controls (n=18)*
A	6	7
B	3	2
C	2 } 7	3 } 8
D	2	3
E	2	3

for colectomy (Table VII); otherwise, the surgical indications were nearly identical between the two groups. It has been suggested that emergency colectomy and postoperative sepsis may predispose to the development of pouchitis.[14] Our review of clinical features, however, found no increased incidence of pouchitis in cases that had had emergency colectomy or postoperative sepsis (Table VII).

As shown in Table VIII, the 13 cases that were judged histologically to be definite ulcerative colitis (category A) were distributed almost evenly between the RP and control groups. By the same token, five cases representing Crohn's disease (category E) by virtue of containing sarcoid like granulomata (evidently overlooked by the original pathologists), as well as 15 cases with varying degrees of overlapping histology (categories B, C, and D), were also distributed almost evenly between the two groups. Finally, there were no statistically significant associations of RP *v* control classification with any of the 23 individual histological features listed in Table IV, including the presence of chronic ileitis.

Discussion

Typical pouchitis has been reported in about 20% of ileal reservoirs, although incidences in different series range from 7–42%, with equal frequency in both pelvic and Kock pouches.[1-3 9-17] By contrast, the syndrome that we have termed refractory pouchitis (RP) is comparatively rare. It may correspond to what the Lahey Clinic group has called 'chronic' pouchitis.[18] Our cases of RP represent only about 3% of the ileal pouches constructed at The Mount Sinai Hospital during the 13 year period studied.

The mean interval from reservoir surgery to onset of pouchitis in our series was 22 months (range 2–72 months). This figure conforms closely to the experience of others. Zuccaro *et al*[19] reported a mean interval of 25 months (range 3–54 months) and Lohmuller *et al*[20] reported a mean interval of 17 months (range 2 days–93 months). There may in fact be some differences in these time intervals and in other pathogenetic factors between Kock pouches and pelvic pouches, but in view of the general clinical similarities between the RP syndromes in these two forms of pouch (Table V), we have felt justified in combining them for the purposes of our analysis.

Because the endoscopical findings in RP often include serpiginous ulcerations, fissures, and ileitis proximal to the pouch, the syndrome has often been attributed to underlying Crohn's disease, the diagnosis of which had presumably been 'missed' both clinically and pathologically up to and including the time of colectomy. There has been no systematic study, however, to discover if this assumption is correct. We therefore sought clinical and pathological features that might test this concept and help us identify risk factors for the development of RP.

The only distinguishing clinical features we could identify in the RP group were male preponderance and an increased frequency of extraintestinal manifestations. We are not aware that a male preponderance has been previously seen in association with any form of pouchitis. Lohmuller *et al*[20] reported that among 671 patients with chronic ulcerative colitis, if preoperative extraintestinal manifestations had been present, pouchitis occurred in 39% of cases, but in only 26% if no extraintestinal manifestations had been present. They also showed by lifetable analysis that the risk of pouchitis at 5 years was 44% if preoperative extraintestinal manifestations had been present *v* 33% if they had been absent. Similarly Becker *et al*[21] reported that if their ulcerative colitis patients had preoperative extraintestinal manifestations, 39% developed pouchitis *v* only 19% among those patients who had not had preoperative extraintestinal manifestations. Indeed, severe systemic manifestations have been reported in the course of pouch ileitis itself.[22]

Blind pathological review of the colectomy specimens showed no correlation between histological category and the presence or absence of RP. For example, colectomy specimens were classified as definite ulcerative colitis (Group A) as frequently in RP as in controls (40% and 39%, respectively). This lack of significant difference between the proportions of RP cases and controls held true for the other histological categories as well, including even patients with definite Crohn's disease (Group E) (RP 13%, control 17%). Our findings are supported by the Mayo Clinic report of no increased incidence of pouchitis in cases of histologically indeterminate colitis compared with cases of more classic ulcerative colitis.[23]

Our review also attempted to discern any correlation between RP and individual histological features, primarily those considered to be indicators of classic Crohn's disease, but we found no such associations. For example, the same proportions of RP and control patients had histological evidence of ileitis on review of their surgical specimens (20% and 17%, respectively). This particular finding supports the finding, based on radiological studies, that the presence of backwash ileitis did not predispose to an increased incidence of pouchitis.[24]

It should be emphasised that even the five

cases classified pathologically as Group E were not histologically compelling examples of classic Crohn's disease apart from the presence of typical granulomata. Granulomata themselves were comparatively infrequent, and other histological features characteristic of Crohn's disease were less conspicuous than usual. Therefore, we are not implying that patients with classic Crohn's disease are necessarily good candidates for reservoir surgery, nor does the recent experience of The Cleveland Clinic support such a view.[25] We are suggesting, however, that the postoperative development of RP is not prima facie evidence for a 'missed' diagnosis of Crohn's disease before and including the time of colectomy.

The cause of RP may be linked to the still unelucidated immunological mechanisms in inflammatory bowel disease that are clinically manifested as extraintestinal complications. Perhaps an immunologically vulnerable subset of inflammatory bowel disease patients, Crohn's disease and ulcerative colitis alike, are prone to develop refractory pouchitis under the influence of certain mechanical, vascular, bacteriological or biochemical factors, or all four that may arise in an ileal reservoir.[26-29] This concept may explain why the inflammatory changes manifested in the clinical syndrome of 'pouchitis' are seen almost exclusively in patients who have had colectomy for inflammatory bowel disease rather than for familial polyposis.[19]

Whatever the pathophysiological basis of RP may be, however, it is worthy of serious study as a 'human experimental model' of inflammatory bowel disease,[30] rather than cavalier dismissal as simply a 'misdiagnosis' of Crohn's disease.

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Exhibit N

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Review article: medical treatment of active Crohn's disease

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SUMMARY

Crohn's disease, a heterogenous inflammatory process that can affect various sites in the gut, presents an ongoing management challenge for the clinician. The treatment of active disease and complications is one of the main goals in the therapy of this disease. New therapies are aimed at delivering the active compounds to the diseased site, reduction or suppression of enteral flora and modulation of more focal targets within the immune response.

The use of antibiotics in the therapy of Crohn's disease is gaining popularity, on the grounds that intestinal bacteria may play a role in the pathogenesis of Crohn's disease lesions. Metronidazole is one of the most widely used antibiotics, especially in the treatment of perianal disease.

Corticosteroids are the mainstays of medical treatment in active Crohn's disease and induce the remission of symptoms in about 60–80% of patients.

The use of immunosuppressive agents, such as cyclosporine and methotrexate, in patients with active disease resistant to standard therapy has gained acceptance in recent years.

With new therapies the outlook for patients with Crohn's disease is more optimistic than it has been for a long time.

INTRODUCTION

Crohn's disease is a heterogenous inflammatory process that can affect various sites of the gut, in extensive or localized form, characterized by different patterns, according to disease location and behaviour (inflammatory, fistulizing or fibrostenotic), which influence the clinical picture.[1] In the natural history of Crohn's disease there is an alternation between relapse phases in which the disease is symptomatic, and remission phases. The treatment of active disease and complications is one of the main goals in the therapy of this disease.

Traditional medical therapies include 5-aminosalicylic acid (5-ASA) and corticosteroids. Newer therapies are aimed at delivering the active compounds to the diseased site, reduction or suppression of enteral flora and modulation of more focal targets within the immune response.

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AMINOSALICYLATES

Large clinical trials have shown that Salazopyrine (SASP), a compound of 5-ASA and sulfapyridine (SP), to be superior to placebo in the treatment of patients with active Crohn's disease.[2, 3] The uncertain efficacy of this drug when the disease is limited to the small intestine and the appearance of side-effects mainly connected with SP, have led to the elaboration of new products comprising exclusively 5-ASA. Several oral preparations of 5-ASA are available with different delivery systems which prevent the absorption of the molecule by the stomach and the proximal portions of the small intestine, allowing the release of the active drug further down the intestinal tract. Salofalk, Pentasa, Claversal and Asacol have been evaluated in many controlled trials to determine their efficacy in treating acute exacerbations of Crohn's disease, generally showing a therapeutic advantage over placebo, but inferior to steroids (Table 1). The relative inefficiency of

Table 1. Controlled trials on efficacy of 5-aminosalicylic acid (5-ASA) in active Crohn's disease

Author	Year	Active drug	Dose of 5-ASA (g/day)	*N*	Period	Control	Therapeutic advantage 5-ASA %
Maier *et al.*[4]	1985	Salofalk	1.5	30	8 weeks	SASP	+ 7
Saveymuttu *et al.*[5]	1986	Pentasa	1.5	12	10 days	Placebo	+ 50
Rasmussen *et al.*[6]	1987	Pentasa	1.5	67	16 weeks	Placebo	+ 10
Mahida & Jewell[7]	1990	Pentasa	1.5	40	6 weeks	Placebo	+ 5
Scholmerich *et al.*[8]	1990	Claversal	2.0	62	24 weeks	Steroid	− 39
Maier *et al.*[9]	1990	Salofalk	3.0	50	12 weeks	Steroid + SASP	− 5.5
Martin *et al.*[10]	1990	Salofalk	3.0	50	12 weeks	Steroid	+ 1
Singleton *et al.*[11]	1993	Pentasa	4.0, 2.0, 1.0	310	16 weeks	Placebo	+ 25, + 6, + 5
Tremaine *et al.*[12]	1994	Asacol	3.2	38	16 weeks	Placebo	+ 23
Gross *et al.*[13]	1995	Salofalk	4.5	34	8 weeks	Steroid	− 16.3
Prantera *et al.*[14]	1999	Asacol	4.0	94	12 weeks	Steroid	− 1
		Asacol microgranular	4.0				+ 18
Thomsen *et al.*[15]	1998	Budesonide	4.0	182	16 weeks	Pentasa	− 26
Colombel *et al.*[16]	1999	Ciprofloxacin	4.0	40	6 weeks	Pentasa	− 1

N, number of patients; Bold type indicates a statistically significant therapeutic advantage; SASP, salazopyrine.

mesalamine could be explained by a variety of drug-related properties:

- the fairly low dosage of 5-ASA administered in the first studies. Only in the most recent trials have doses of 4 g and over been employed;
- the use of 5-ASAs with different delivery systems that release the active substance in different intestinal sites;
- the probable topical action of mesalamine;
- the eventual faecal loss of the drug, following accelerated transit or an extensive bowel resection. Some aspects that may explain the inefficacy could be related to the disease:
- Crohn's disease affects different locations of the intestine with different disease behaviour;
- Crohn's disease often involves the deep layers of the gut, and 5-ASA, because of its topical action, may be more efficacious when the lesions are localized in the superficial layer.

ANTIBIOTICS

The use of antibiotics in the therapy of Crohn's disease is today more popular than in the past, on the grounds that intestinal bacteria may play a role in the pathogenesis of Crohn's disease lesions.[17, 18] Some controlled studies have shown their efficacy, as an alternative to, or in combination with, corticosteroids.

Metronidazole

Among several antibiotics, metronidazole, which is active against anaerobic bacteria and some parasites, is the most widely used, especially in the treatment of perianal disease. The first controlled study, performed in 22 patients treated with SASP or steroids plus metronidazole or placebo, did not demonstrate any improvement in patients on the antibiotic, although some positive results were shown in colitis.[19] In the Cooperative Crohn's disease Study in Sweden, a randomized crossover trial in which metronidazole was compared with SASP, the drugs were equally effective only when the colon was involved.[20] In a trial from Birmingham, metronidazole alone or in combination with cotrimoxazole had little therapeutic value in active Crohn's disease.[21] A Canadian study also showed that metronidazole was effective when the colon was involved.[22] The positive results obtained with metronidazole in Crohn's colitis or ileocolitis but not in the small bowel, might be due to the higher concentration of anaerobic bacteria in colitis, and the efficacy of this antibiotic seems to be correlated with luminal *Bacteroides* concentration.

Ciprofloxacin

Another antibiotic used in Crohn's disease is ciprofloxacin, a quinolone derivative with a selective suppressive

effect on the intestinal flora. *Escherichia coli* and Enterobacteriaceae are especially sensitive to this antibiotic, which, on the other hand, does not particularly affect anaerobic bacteria.

Five randomized controlled studies have employed ciprofloxacin, alone or associated with metronidazole, in the treatment of active Crohn's disease, reporting interesting results.[16, 23–26] In one of these trials from Italy, 41 patients were randomized to receive a combination of ciprofloxacin and metronidazole at a dosage of 1 g/day or methylprednisolone, for a period of 12 weeks.[24] Ten of 22 patients on antibiotics (45%) and 12 of those treated with steroids (63%) reached clinical remission at the end of the study. Despite a high incidence of adverse events responsible for withdrawl of the patients from the trial in the antibiotic group (27%), the association of ciprofloxacin and metronidazole was shown to be an effective alternative to corticosteroids in the therapy of acute Crohn's disease. Another controlled study has shown that ciprofloxacin is as effective as mesalamine in treating 40 patients with mild to moderate flare-up of Crohn's disease.[16]

CORTICOSTEROIDS

Corticosteroids are the mainstays of medical treatment in active Crohn's disease and induce the remission of symptoms in a high percentage of patients, about 60–80%.[2, 3] Because of the side-effects produced by systemic steroids, new glucocorticoid derivatives, such as budesonide, which act locally in the mucosa and have little systemic activity, have recently been introduced.

Budesonide

The major advantage of controlled-release budesonide formulation is its first-pass rapid metabolism by the liver that results in inactive metabolites and consequently in reduced or absent systemic side-effects. The clinical value of budesonide in active Crohn's disease has been established in several controlled studies.[27–31] This drug, at a dosage of 9 mg daily, has been shown to have an efficacy which is comparable to prednisone or methylprednisolone, 40 mg/day,[27, 30, 31] and superior to placebo[28] in the therapy of active Crohn's disease involving the distal ileum and/or right colon. Fewer steroid-related side-effects were reported in the patients on budesonide compared to patients treated with systemic corticosteroids.

IMMUNOSUPPRESSIVE THERAPY

Immunosuppressive therapy for the treatment of Crohn's disease has gained increasing acceptance in recent years. The immunosuppressive agents used in patients with active disease resistant to standard therapy are cyclosporin and methotrexate, which act more rapidly than azathioprine and 6-mercaptopurine.

Cyclosporine

Cyclosporine is a potent immunosuppressant, only recently introduced into the field of Crohn's disease. Some anecdotal reports and only one randomized placebo-controlled trial, in which cyclosporin was used at a median dosage of 7.6 mg/kg/day by mouth, have shown encouraging but not impressive results in the use of this drug for active Crohn's disease refractory to conventional agents.[32] Three other randomized controlled studies, each employing oral cyclosporin doses of 5 mg/kg per day or less in active disease found no benefit.[33–35] The risk of nephrotoxicity is dose dependent, increasing with a dosage above 5 mg/kg daily. However, this side-effect was not confirmed in a study that reported the follow-up of 1663 patients treated with cyclosporin at doses of between 3 and 6 mg/kg per day.[36]

Methotrexate

The efficacy of methotrexate in inflammatory conditions such as rheumatoid arthritis is well known. Its effects in refractory Crohn's disease were first reported by Kozarek *et al.* in an open study 12 years ago.[37] Subsequently, two randomized controlled studies have compared methotrexate to placebo in the induction of remission.[38, 39] The largest trial involved 141 patients with chronic active Crohn's disease treated with parenteral methotrexate at a dosage of 25 mg per week against placebo.[38] Thirty-nine percent of patients taking methotrexate were able to discontinue steroids and entered remission, compared with 19% on placebo. In a more recent study methotrexate given orally at a weekly dose of 12.5 mg was no more effective than placebo in inducing remission.[39]

CONCLUSION

Crohn's disease presents an ongoing management challenge for the clinician. With new therapies the

outlook for patients with Crohn's disease is more optimistic than it has been for a long time. The medical treatment of active phases is often a wise combination of old and new drugs.

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